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VITAMIN A IN OLEO OIL AND OLEO STEARIN[1]

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INTRODUCTION

Oleo oil is the name given to the fluid portion obtained by pressing rendered beef fat at a temperature approximating 90° F., the solid residue being designated oleo stearin. In commercial practice, yields approximating 70 per cent of oleo oil and 30 per cent of oleo stearin are ordinarily obtained. Oleo oil is usually graded as No. 1, No. 2, No. 3, and yellow oil, the latter being prepared from fats derived from grass-fed cattle.

The production of oleo oil in this country in 1924 (*7*)[2] amounted to 156,334,129 pounds, of which 49,701,460 pounds entered into domestic consumption. Oleo oil is used chiefly in the manufacture of oleomargarine, and oleo stearin in the preparation of baking compounds.

The most important known sources of vitamin A among our food products are butter, eggs, liver, kidneys, milk, and green vegetables. Beef fat and oleo oil have been found to contain a fair amount of this vitamin, but relatively few investigations have been carried on with these two products. In view of the importance of oleo oil and oleo stearin as food, it has seemed desirable to make a systematic study of the vitamin content of these fats.

PREVIOUS INVESTIGATIONS

Osborne and Mendel (*4*) found that 18 per cent of beef fat, rendered in the laboratory, furnished nearly sufficient vitamin A for normal growth in rats. Beef oil, prepared by the fractional crystallization of beef fat from alcohol, was found to be rich in vitamin A, but the crystalline fractions with high melting points proved to be inactive.

Halliburton and Drummond (*3*) found that a ration containing 20 per cent of oleo oil was inadequate for normal growth in rats.

Steenbock, Boutwell, and Kent (*5*) determined the vitamin A value of three samples of oleo oil when used to the extent of 5 per cent in the ration. A sample of "prime" oleo oil induced but little growth in rats, but fair results were obtained from "extra" oleo oil and from an ungraded oleo oil.

Drummond and Coward (*1*) obtained only fair growth in rats when the ration contained 15 per cent of mutton fat as the source of vitamin A.

[1] Received for publication July 6, 1925; issued February, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 416.

Steenbock, Sell, and Buell (*6*) studied the vitamin A content of beef fat as related to the intensity of the yellow color of the product. They found that deep-yellow fat was much richer in vitamin than the lighter-colored fat. In some instances 5 per cent of yellow fat induced good growth in rats, whereas a like proportion of light-colored fat was inadequate.

EXPERIMENTAL PROCEDURE

In this investigation the vitamin A content of the samples of oleo oil and oleo stearin was determined by feeding tests with young albino rats, the fat constituting the only known source of vitamin A in an otherwise adequate ration. The rats used were grown in the animal laboratory of the Bureau of Animal Industry of the United States Department of Agriculture. Litters of more than eight rats were reduced to that number on the day of birth, and litters of fewer than six rats were discarded. Only young rats that weighed at least 40 grams each when 30 days of age were used for experimental purposes.

The basal ration was made up according to the following formula:

	Per cent
Protein (N x 6.25)	20
Fat	10
Ash mixture	4
Dried yeast	10
Starch to make	100

The protein consisted of crude dried casein which had been heated three or four days in a current of air at a temperature of 115° C. in order to destroy vitamin A. Fat was supplied in the form of hardened cottonseed oil. The ash mixture was made up according to a formula by Drummond and Watson (*2, p. 237*). Dried bakers' yeast and cassava starch made up the rest of the ration. The constituents other than casein were found to be free from vitamin A. The fat was melted and thoroughly mixed with the other constituents, a total quantity of 1,000 grams of the ration being prepared at one time. The experimental rations which contained oleo oil or oleo stearin were made up according to the same formula as the basal ration, except that the fat content was increased to 20 per cent. When less than this proportion of oleo oil or oleo stearin was added to a ration, hardened cottonseed oil was used to bring the fat content up to 20 per cent.

Each ration was fed to four rats, usually equally divided as regards sex. Each rat was kept in a cylindrical wire cage with a separate pan bottom which was covered with a sheet of blotting paper and a flat wire screen. The ration was supplied in a self-feeder, and water in a glass siphon-fountain. The rats were weighed regularly twice each week, and they were examined frequently for signs of ophthalmia.

Through the courtesy of the meat inspection division of the Bureau of Animal Industry, the samples of oleo oil and oleo stearin which are described in Table I were obtained from officially inspected establishments located in some of the more important meat-packing centers. In some instances, the establishments used trade or other names to designate the different grades of oleo oil, but for the sake of uniformity, such samples have been given the usual grade numbers.

TABLE I.—*Description of samples of oleo oil and oleo stearin tested for vitamin A*

Sample No.	Description of sample	Source		Date collected
		City	Establishment *	
				1924
1125	No. 1 oleo oil	Chicago, Ill.	A	June 12
1126	No. 2 oleo oil	do	A	Do.
1127	No. 3 oleo oil	do	A	Do.
1176	Yellow oleo oil	do	A	Sept. 10
1172	do	do	B	Sept. 8
1173	do	do	C	Do.
1177	do	do	D	Sept. 16
1178	do	do	E	Do.
1115	No. 2 oleo oil	Denver, Colo.	A	May 28
1110	Yellow oleo oil	Fort Worth, Tex.	A	May 21
1111	No. 2 oleo oil	do	A	Do.
1120	Mutton oleo oil	do	A	June 4
1052	No. 1 oleo oil	Kansas City, Kans.	A	Feb. 20
1053	No. 1 oleo stearin	do	A	Do.
1054	No. 2 oleo oil	do	A	Do.
1055	No. 2 oleo stearin	do	A	Do.
1056	No. 3 oleo oil	do	A	Do.
1057	No. 3 oleo stearin	do	A	Do.
1076	Yellow oleo oil	do	A	Mar. 12
1077	Yellow oleo stearin	do	A	Do.
1059	No. 1 oleo oil	do	B	Feb. 20
1060	No. 1 oleo stearin	do	B	Do.
1061	No. 2 oleo oil	do	B	Do.
1062	No. 2 oleo stearin	do	B	Do.
1078	No. 3 oleo oil	do	B	Mar. 12
1079	No. 3 oleo stearin	do	B	Do.
1080	Yellow oleo oil	do	B	Do.
1081	Yellow oleo stearin	do	B	Do.
1113	No. 1 oleo oil	New York, N. Y.	A	May 26
1124	No. 2 oleo oil	South St. Paul, Minn.	A	June 14
1141	No. 1 oleo oil	do	A	June 27
1142	Yellow oleo oil	do	A	Do.

* The letters used to designate the several establishments have been assigned arbitrarily without reference to the official numbers which these establishments bear for the purpose of meat inspection.

FEEDING TESTS WITH BASAL RATIONS

In the experiments reported in this paper, each ration containing oleo oil or oleo stearin was fed to a group of rats from the start, without a preliminary feeding test with a vitamin-A-free basal ration, as is the practice followed by many investigators. The procedure which the writers have followed requires that proper allowance be made for the probable growth that the rats fed the rations containing oleo oil or oleo stearin would have made had they been fed the basal ration alone. For this purpose several groups of rats were fed basal rations alone during the course of this investigation, these rations containing a sample of each lot of purified casein that had been used in the preparation of the experimental rations. The results of the feeding tests with the basal rations are shown in Figures 1 and 2.

Referring to Figures 1 and 2, it might appear from the growth made by the several groups of rats that there were fairly wide differences in the vitamin A content of the several basal rations and that some of them contained appreciable amounts of this vitamin. It is believed, however, that the basal rations were practically free from vitamin A and that the considerable differences in growth were due to differences in the reserve stores of vitamin A in the tissues of the young rats at the start of the experiments.

During the course of the experiments the breeding rats were at first fed a ration very low in vitamin A, but on account of unsatisfactory reproduction the vitamin A content had to be increased materially. Reproduction soon became normal but it was found that the young rats made too much growth when fed a vitamin-A-free basal ration, and the amount of this vitamin in the ration fed

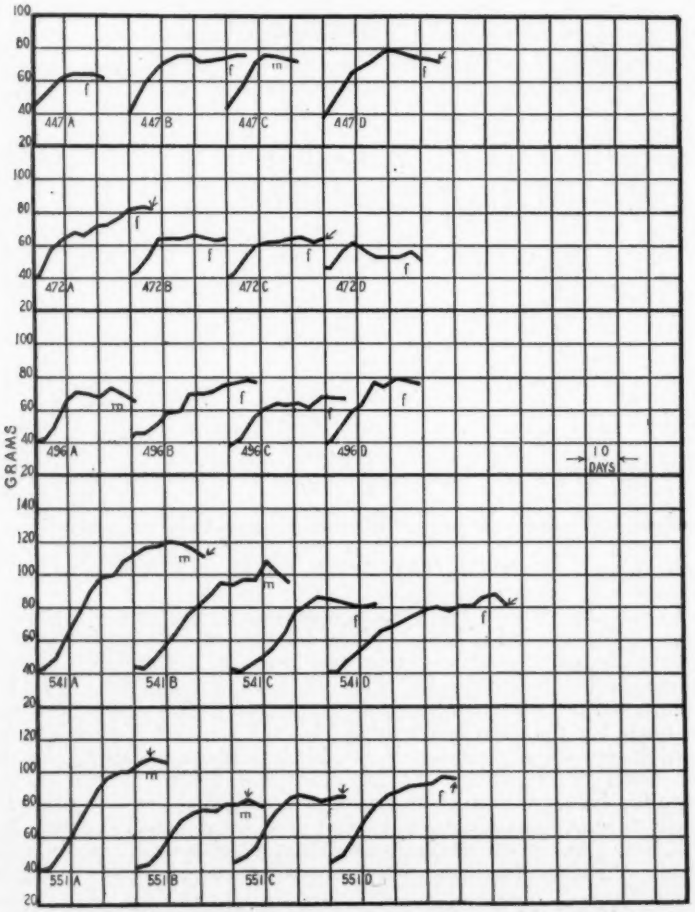

FIG. 1.—Growth curves of five groups of rats that were fed basal rations alone during the course of this investigation. The arrows indicate the development of ophthalmia. The sex of each rat is indicated by the letter *m* or *f*

the breeding rats was therefore materially reduced. Referring to Figures 1 and 2, rats Nos. 447, 472, and 496 were produced during the first interval noted above; Nos. 541, 551, 552, 553, and 589 during the second interval; and No. 599 during the last period. Thus it appears that the growth made by the rats fed the basal rations bears

a direct relation to the vitamin A content of the ration fed to the mother rats. This is in harmony with the experience of other investigators.

In the interpretation of the results of the feeding tests with the rations containing oleo oil or oleo stearin, the growth made by each group of rats should be compared with that made by the nearest-numbered group of rats that had been fed a basal ration.

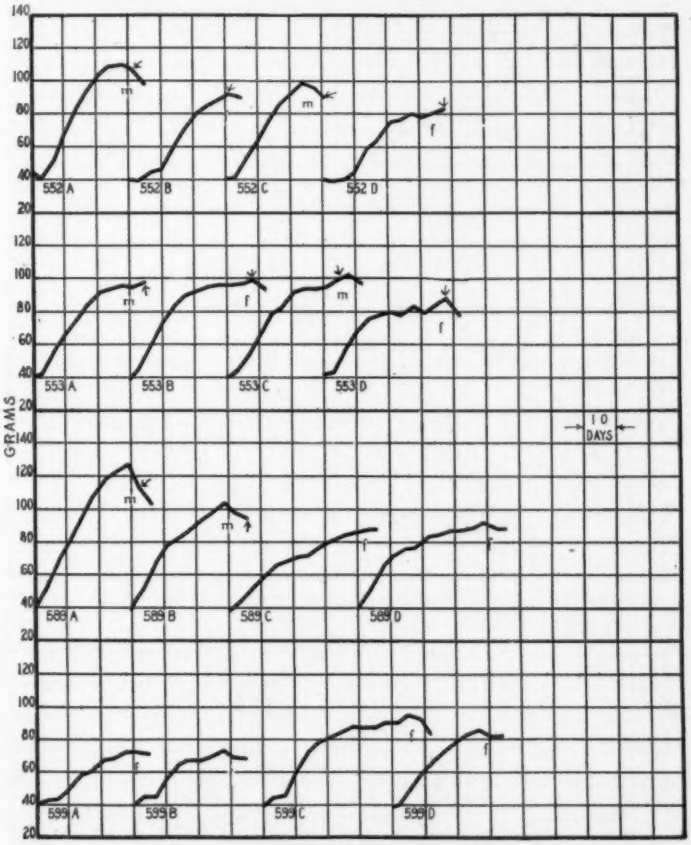

FIG. 2.—Growth curves of four groups of rats that were fed basal rations alone during the course of this investigation. The arrows indicate the development of ophthalmia. The letters *m* and *f* indicate the sex of the rats

FEEDING TESTS WITH OLEO OIL AND OLEO STEARIN

SAMPLES FROM CHICAGO

In Figures 3 to 6, inclusive, are shown the growth curves of rats that were fed samples of No. 1, No. 2, No. 3, and yellow oleo oil, respectively, from establishment A. Each of the first three rations contained 20 per cent of oleo oil, but the fourth contained only 10

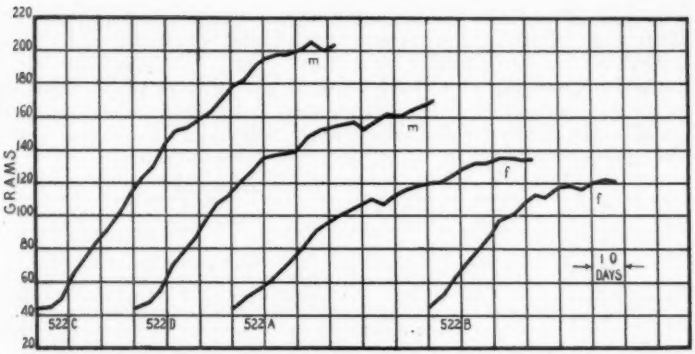

FIG. 3.—Growth curves of rats fed a ration containing 20 per cent of No. 1 oleo oil, from establishment A, Chicago, Ill.

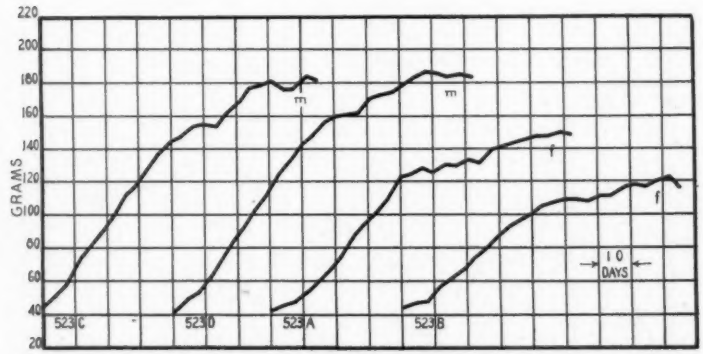

FIG. 4.—Growth curves of rats fed a ration containing 20 per cent of No. 2 oleo oil, from establishment A, Chicago, Ill.

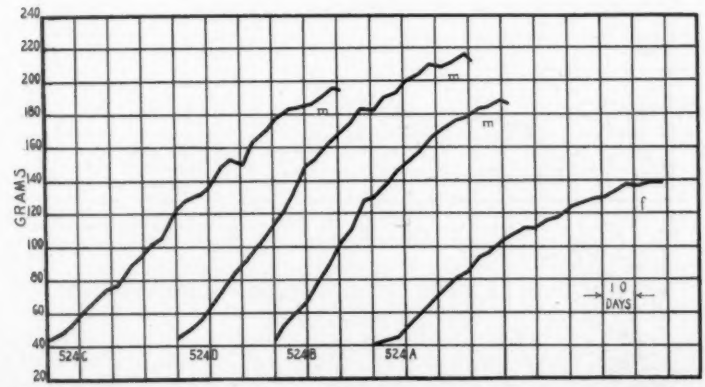

FIG. 5.—Growth curves of rats fed a ration containing 20 per cent of No. 3 oleo oil, from establishment A, Chicago, Ill.

per cent. The rats that were fed the samples of No. 1 and No. 2 oleo oil, respectively, made approximately the same growth; those getting the No. 3 oil made slightly better growth; whereas those that were fed the yellow oil made the most satisfactory growth of

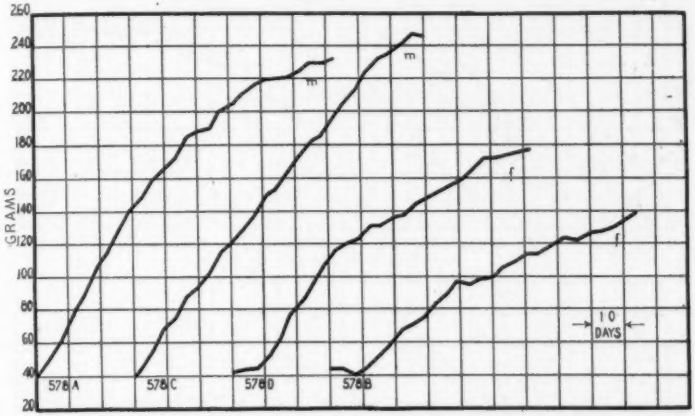

FIG. 6.—Growth curves of rats fed a ration containing 10 per cent of yellow oleo oil, from establishment A, Chicago, Ill.

all. The rats getting the No. 3 oleo oil made very nearly normal growth, and those fed the yellow oil grew at a normal rate.

The results of these tests indicate that the samples of No. 1 and No. 2 oleo oil had approximately the same vitamin-A value, that the No. 3 oil had a slightly higher value, and that the yellow oil was decidedly the richest in vitamin A.

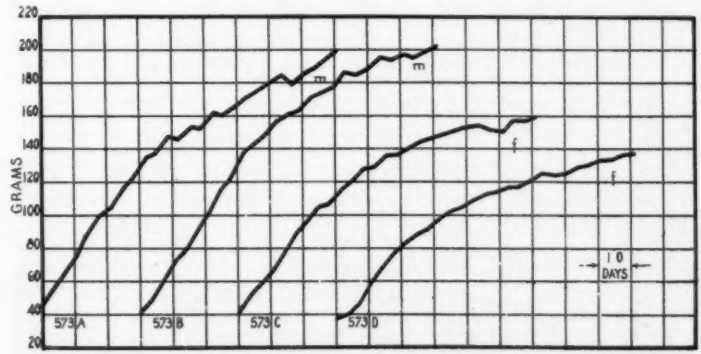

FIG. 7.—Growth curves of rats fed a ration containing 10 per cent of yellow oleo oil, from establishment B, Chicago, Ill.

In Figures 7 to 10, inclusive, are shown the results of feeding tests with samples of yellow oleo oil from establishments B, C, D, and E, each ration containing 10 per cent of oleo oil. The rats getting the yellow oil from establishment B made very good, though not quite

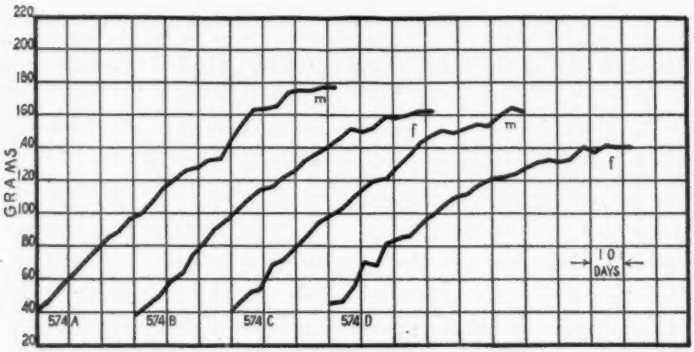

FIG. 8.—Growth curves of rats fed a ration containing 10 per cent of yellow oleo oil, from establishment C, Chicago, Ill.

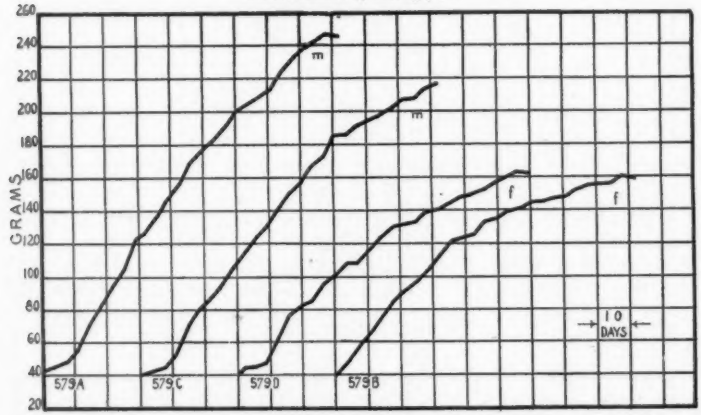

FIG. 9.—Growth curves of rats fed a ration containing 10 per cent of yellow oleo oil, from establishment D, Chicago, Ill.

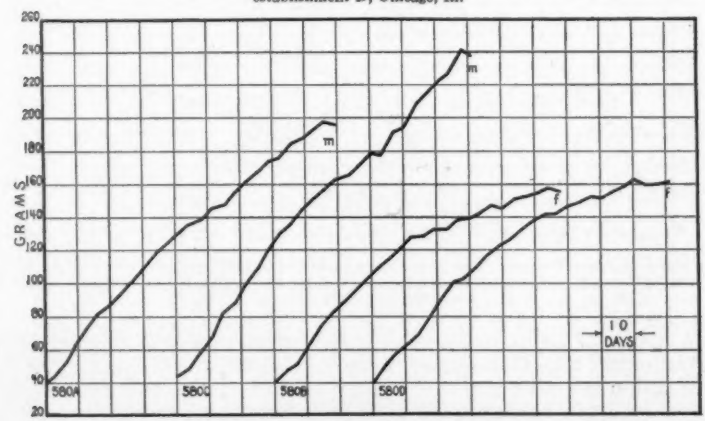

FIG. 10.—Growth curves of rats fed a ration containing 10 per cent of yellow oleo oil, from establishment E, Chicago, Ill.

normal, growth (fig. 7). Those fed the yellow oil from establishment C made nearly as satisfactory growth (fig. 8). The two groups that received the yellow oil from establishments D and E grew at a normal rate (figs. 9 and 10).

The results of these tests indicate that the five samples of yellow oleo oil were much richer in vitamin A than any other sample of oleo oil obtained in Chicago.

SAMPLE FROM DENVER

Only one sample of No. 2 oleo oil was obtained in Denver. The growth curves of rats fed a ration containing 20 per cent of this oil are shown in Figure 11. This group made normal growth. On comparing these growth curves with those in Figure 4 it is apparent that the sample of No. 2 oleo oil obtained in Denver was somewhat richer in vitamin A than the sample of the same grade of oil obtained in Chicago.

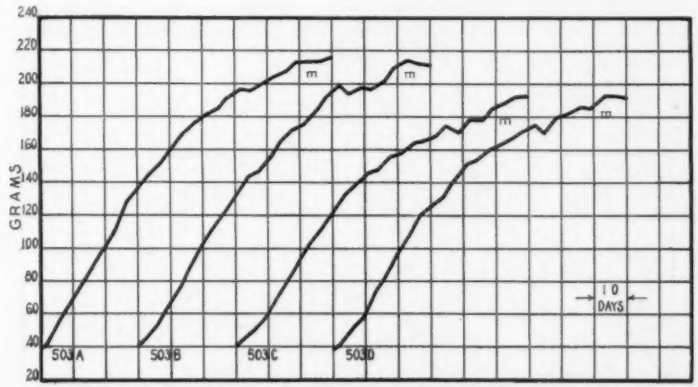

FIG. 11.—Growth curves of rats fed a ration containing 20 per cent of No. 2 oleo oil, from establishment A, Denver, Colo.

SAMPLES FROM FORT WORTH

In Figures 12, 13, and 14 are shown the results of feeding tests with rations each containing 20 per cent of yellow, No. 2, and mutton oleo oil, respectively. The rats that were fed the yellow and the No. 2 oleo oil made normal growth, and those getting the mutton oleo oil grew at a slightly subnormal rate.

The results of these tests indicate no considerable differences in the vitamin content of the three samples of oleo oil, but it is possible that differences would have become apparent had tests been carried on with rations containing 10 per cent of each sample.

SAMPLES FROM KANSAS CITY

In Figures 15 to 18, inclusive, are shown the results of feeding tests with samples of No. 1, No. 2, No. 3, and yellow oleo oil, and the same grades of oleo stearin, from establishment A. In each figure the upper set of curves represents the growth made by the rats fed

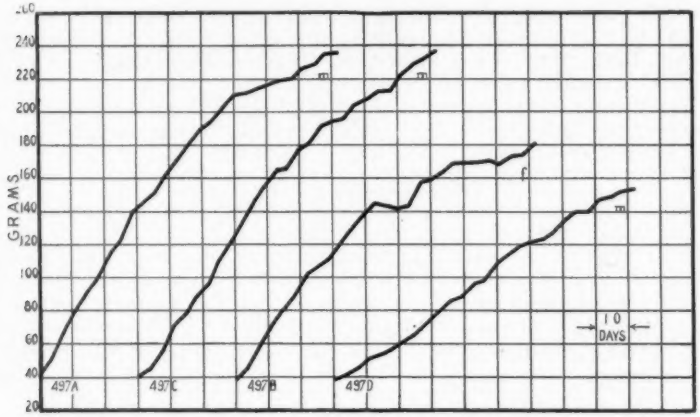

FIG. 12.—Growth curves of rats fed a ration containing 20 per cent of yellow oleo oil, from establishment A, Fort Worth, Tex.

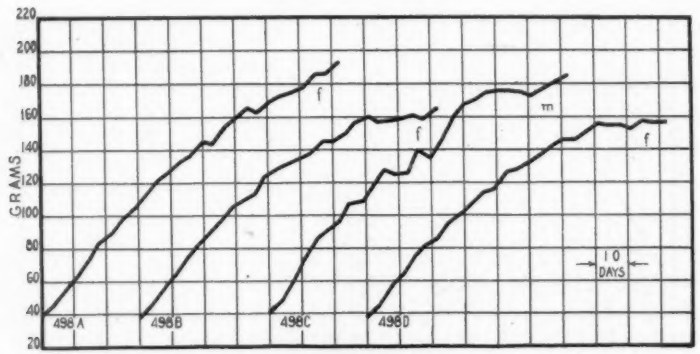

FIG. 13.—Growth curves of rats fed a ration containing 20 per cent of No. 2 oleo oil, from establishment A, Fort Worth, Tex.

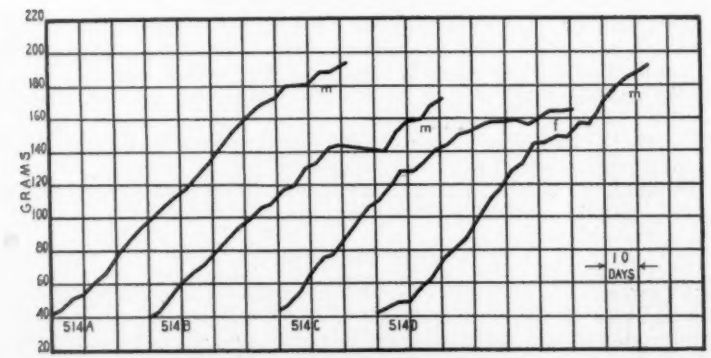

FIG. 14.—Growth curves of rats fed a ration containing 20 per cent of mutton oleo oil, from establishment A, Fort Worth, Tex.

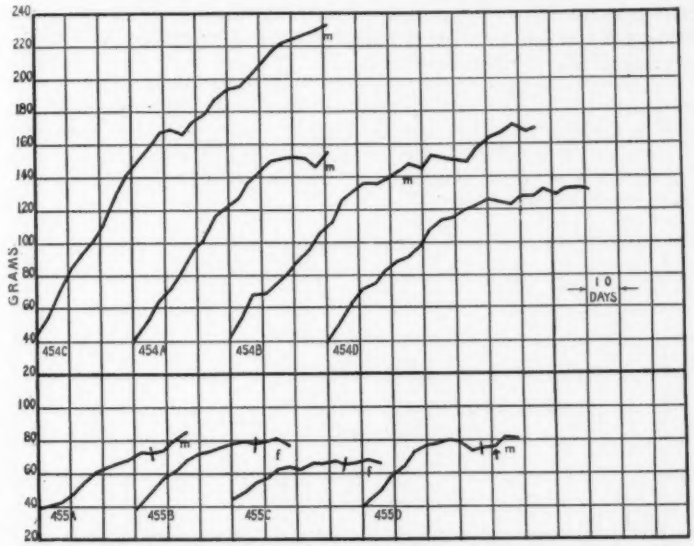

FIG. 15.—Growth curves of two groups of rats fed rations containing 20 per cent of No. 1 oleo oil in one case and 20 per cent of No. 1 oleo stearin in the other. The upper group was fed the oleo oil and the lower the oleo stearin. The short lines intersecting the lower curves indicate the starting of the daily administration of a small quantity of cod-liver oil. The arrow indicates ophthalmia. (The oleo oil and oleo stearin were from establishment A, Kansas City, Kans.)

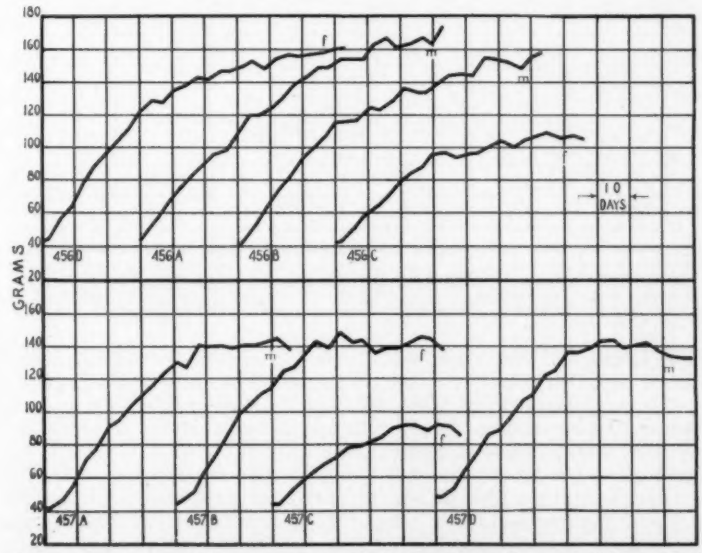

FIG. 16.—Growth curves of two groups of rats fed rations containing 20 per cent of No. 2 oleo oil in one case and 20 per cent of No. 2 oleo stearin in the other. The upper group was fed the oleo oil and the lower group the oleo stearin. (The oleo oil and oleo stearin were from establishment A, Kansas City, Kans.)

a ration containing 20 per cent of oleo oil, the lower set the growth made by those fed a ration containing a like proportion of the corresponding grade of oleo stearin.

The results of these tests indicate that the samples of No. 1 and No. 2 oleo oil had approximately the same vitamin A content, that the No. 3 oleo oil had a slightly higher value, and that the yellow oil was much the richest in this vitamin. The rats getting the yellow oil made normal growth, but those fed the other grades of oil grew at slightly subnormal rates.

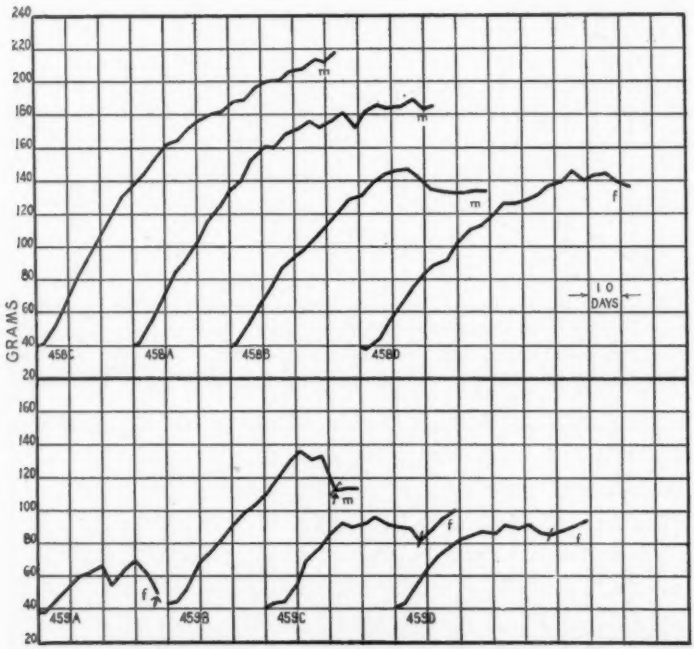

FIG. 17.—Growth curves of two groups of rats fed rations containing 20 per cent of No. 3 oleo oil in one case and 20 per cent of No. 3 oleo stearin in the other. The upper group was fed the oleo oil and the lower group the oleo stearin. The short crosslines intersecting several of the lower curves indicate the starting of the daily administration of a small quantity of cod-liver oil. The arrow indicates ophthalmia. (The oleo oil and oleo stearin were from establishment A, Kansas City, Kans.)

The samples of No. 1 and No. 3 oleo stearin from establishment A (figs. 15 and 17) were very poor in vitamin A, and the samples of No. 2 and yellow stearin (figs. 16 and 18) contained a fair amount. In every instance the sample of oleo oil was much richer in vitamin A than the corresponding sample of oleo stearin.

In Figures 19 to 22, inclusive, are shown the results of feeding tests with samples of No. 1, No. 2, No. 3, and yellow oleo oil, and the corresponding grades of oleo stearin, from establishment B in Kansas City. Each ration contained 20 per cent of oleo oil or oleo stearin.

The results of these tests indicate that the No 1 oleo oil was the poorest in vitamin A, and that the yellow oleo oil was the richest.

The rats that were fed the No. 2 and the yellow oil made normal growth, and those that were fed the No. 3 oil made very nearly normal growth.

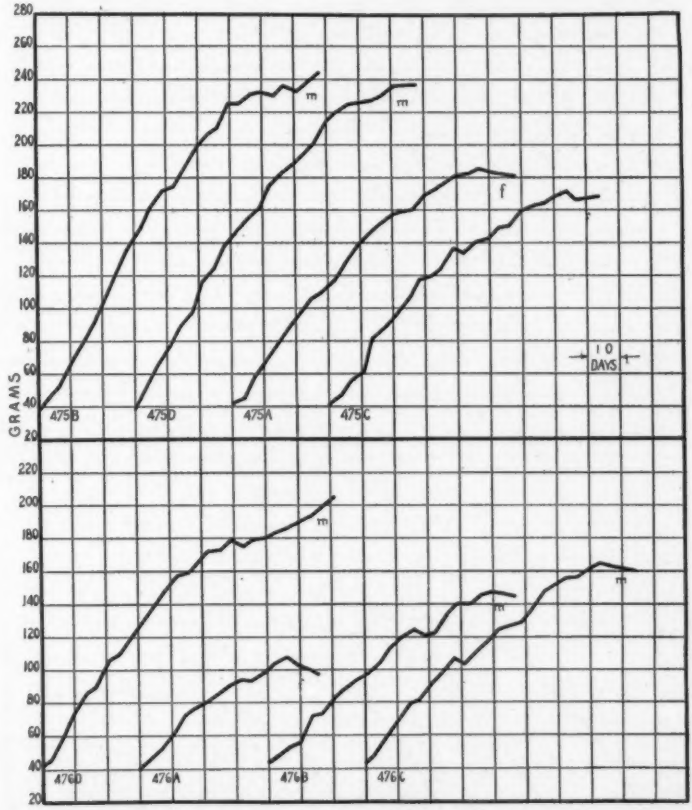

FIG. 18.—Growth curves of two groups of rats fed rations containing 20 per cent of yellow oleo oil in one case and 20 per cent of yellow oleo stearin in the other. The upper group was fed the oleo oil and the lower group the oleo stearin. (The oleo oil and oleo stearin were from establishment A, Kansas City, Kans.)

The samples of No. 1 and No. 2 oleo stearin from establishment B were very poor in vitamin A, the No. 3 stearin contained a fair amount, and the yellow stearin was the richest in this vitamin, comparing favorably with the sample of No. 1 oleo oil from the same establishment. In each case the vitamin A content of a sample of oleo oil obtained from this establishment was much richer in vitamin A than the corresponding sample of oleo stearin.

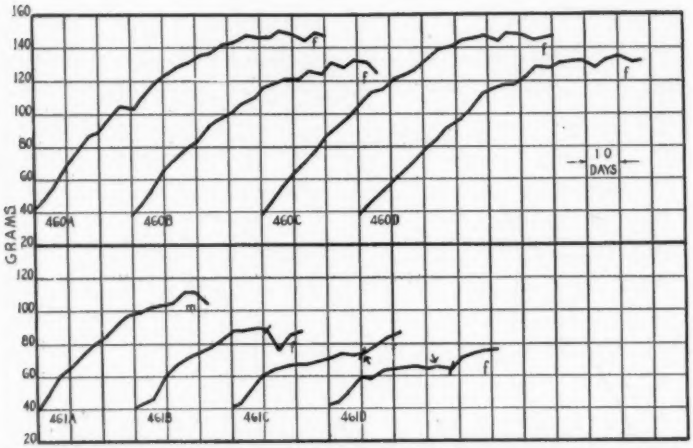

FIG. 19.—Growth curves of two groups of rats fed rations containing 20 per cent of No. 1 oleo oil in one case and 20 per cent of No. 1 oleo stearin in the other. The upper group was fed the oleo oil and the lower group the oleo stearin. The short lines intersecting several of the lower curves indicate the starting of the daily administration of a small quantity of cod-liver oil. The arrow indicates ophthalmia. (The oleo oil and oleo stearin were from establishment B, Kansas City, Kans.)

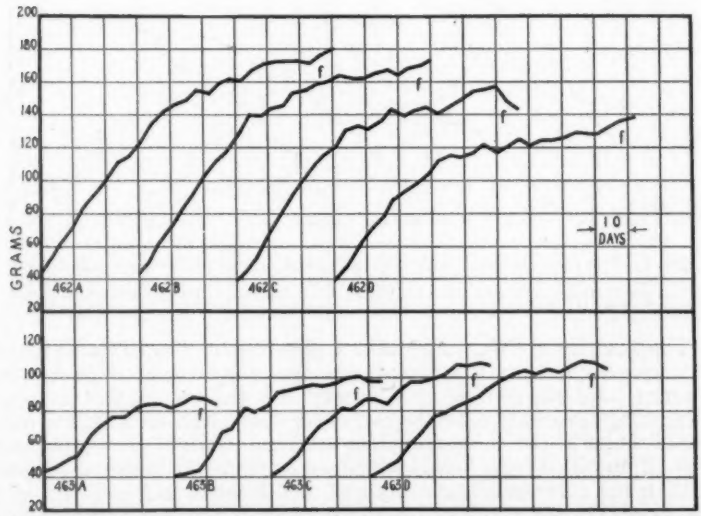

FIG. 20.—Growth curves of two groups of rats fed rations containing 20 per cent of No. 2 oleo oil in one case and 20 per cent of No. 2 oleo stearin in the other. The upper group received the oleo oil and the lower group the oleo stearin. (The oleo oil and oleo stearin were from establishment B, Kansas City, Kans.)

SAMPLE FROM NEW YORK

In Figure 23 are shown the growth curves of rats that were fed a ration containing 20 per cent of No. 1 oleo oil. The rats made fair though somewhat subnormal growth. This sample of oleo oil has approximately the same vitamin A content as samples of the same grade of oleo oil obtained from the other establishments.

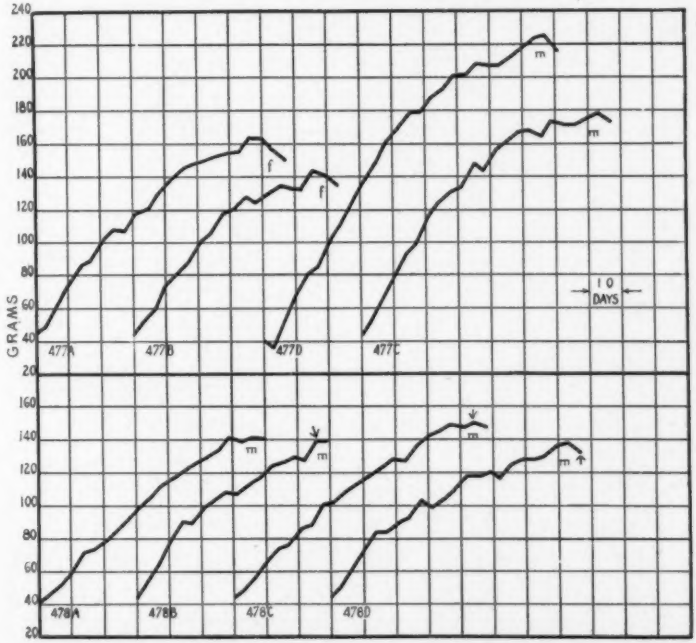

FIG. 21.—Growth curves of two groups of rats fed rations containing 20 per cent of No. 3 oleo oil in one case and 20 per cent of No. 3 oleo stearin in the other. The upper group received the oleo oil and the lower group the oleo stearin. The arrow indicates ophthalmia. (The oleo oil and oleo stearin were from establishment B, Kansas City, Kans.)

SAMPLES FROM SOUTH ST. PAUL

In Figures 24, 25, and 26 are shown the results of feeding tests with samples of No. 1, No. 2, and yellow oleo oil, each group being fed a ration containing 20 per cent of oleo oil. The results indicate that the samples of No. 1 and No. 2 oleo oil had approximately the same vitamin A content, and that the yellow oil had a slightly higher value, the rats getting the latter oil making nearly, though not quite, normal growth.

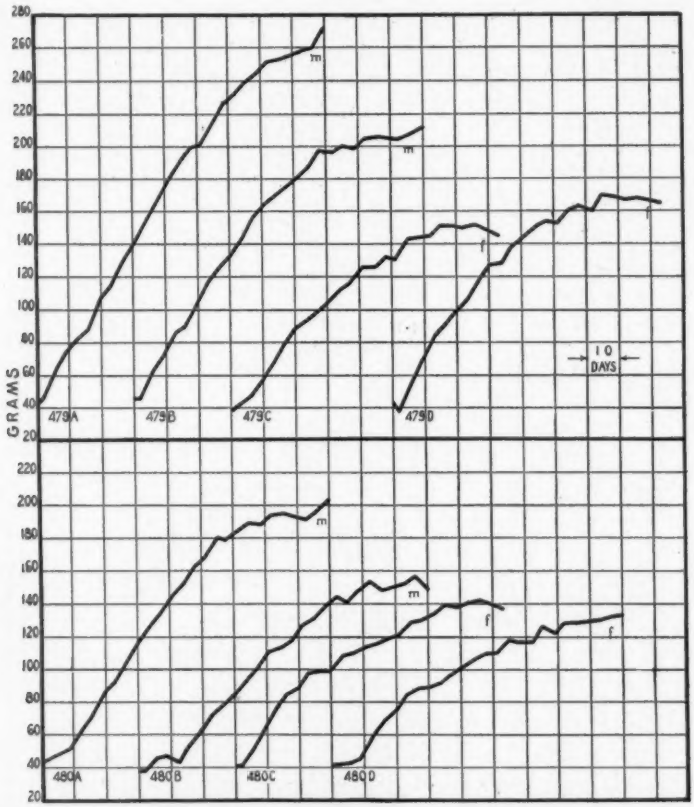

FIG. 22.—Growth curves of two groups of rats fed rations containing 20 per cent of yellow oleo oil in one case and 20 per cent of yellow oleo stearin in the other. The upper group received the oleo oil and the lower group the oleo stearin. (The oleo oil and the oleo stearin were from establishment B, Kansas City, Kans.)

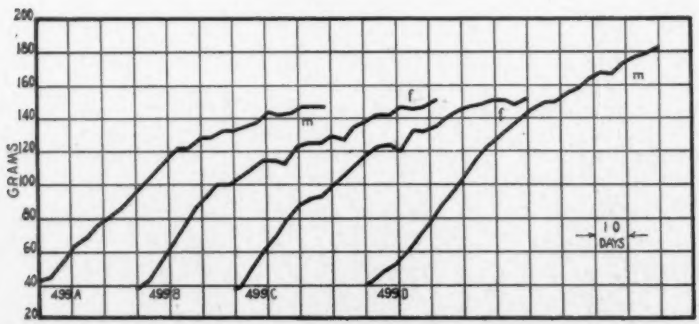

FIG. 23.—Growth curves of rats fed a ration containing 20 per cent of No. 1 oleo oil from establishment A, New York City

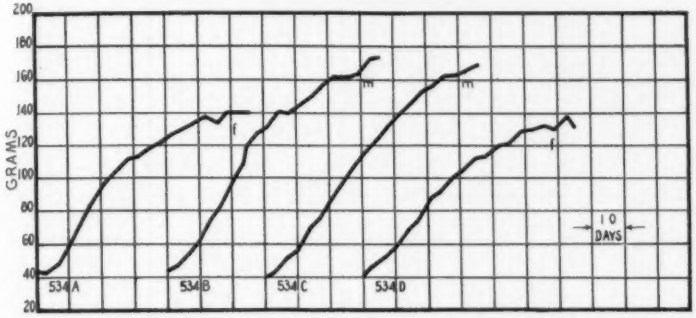

FIG. 24.—Growth curves of rats fed a ration containing 20 per cent of No. 1 oleo oil, from establishment A, South St. Paul, Minn.

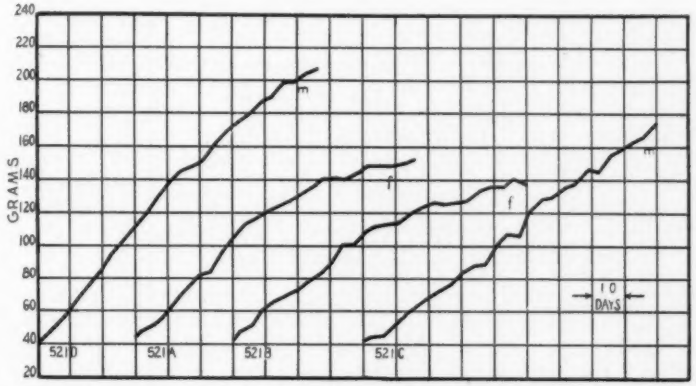

FIG. 25.—Growth curves of rats fed a ration containing 20 per cent of No. 2 oleo oil, from establishment A, South St. Paul, Minn.

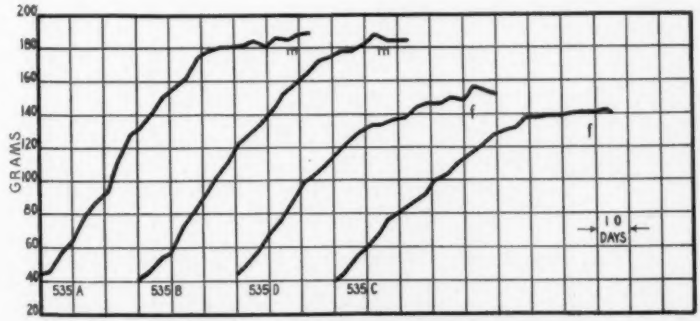

FIG. 26.—Growth curves of rats fed a ration containing 20 per cent of yellow oleo oil, from establishment A, South St. Paul, Minn.

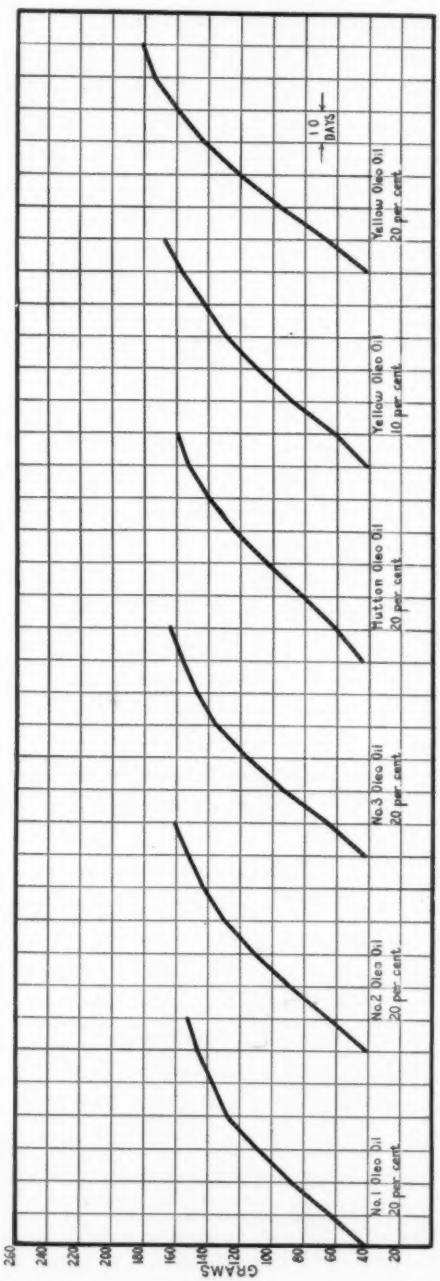

FIG. 27.—Composite growth curves of rats that were fed rations containing No. 1, No. 2, No. 3, mutton, and yellow oleo oils, respectively

SUMMARY AND DISCUSSION

In this paper are reported the results of a study of the vitamin A content of 24 samples of oleo oil, and 8 samples of oleo stearin, collected from commercial meat-packing plants in New York, Chicago, Kansas City, Denver, Fort Worth, and South St. Paul.

The vitamin A content of each sample of oleo oil or oleo stearin was determined by feeding tests with young albino rats, the oil or stearin constituting the only known source of this vitamin in an otherwise adequate ration.

Five samples of No. 1 oleo oil were found to have approximately the same vitamin A content, 20 per cent of this grade of oil in a ration being sufficient for moderate but not normal growth.

Six samples of No. 2 oleo oil were tested for vitamin A. When used to the extent of 20 per cent in a ration, each of three samples furnished sufficient vitamin A for normal growth, but the other samples had somewhat lower values.

Three samples of No. 3 oleo oil were found to have approximately the same vitamin A content. Rations containing 20 per cent of this grade of oil were somewhat inadequate for normal growth.

Nine samples of yellow oleo oil were tested for vitamin A. Of five samples that were fed in rations containing 10 per cent of this grade of oil, three induced normal growth, and two very nearly normal growth. Four samples of yellow oil were fed in rations containing 20 per cent of the oil, and of these, three samples furnished sufficient vitamin A for normal growth and one for moderate growth.

One sample of mutton oleo oil, when it constituted 20 per cent of the ration, induced very nearly, but not quite, normal growth.

In general, it was found that the yellow oleo oil was much the richest in vitamin A; that the No. 2, No. 3, and mutton oleo oil had approximately the same value; and that the No. 1 oleo oil was the poorest in vitamin A.

Eight samples of oleo stearin—two each of No. 1, No. 2, No. 3, and yellow stearin—were tested for vitamin A. Both samples of No. 1 stearin were found to be very poor in vitamin A; one sample each of No. 2 and No. 3 stearin were also very poor in this vitamin, but each of the other samples contained a fair amount; and each sample of yellow stearin was richer in vitamin A than any sample of the other grades of oleo stearin, comparing favorably with some of the samples of No. 1 oleo oil.

Although the yellow oleo oil was found to be richer in vitamin A than the other grades of oil, which were lighter in color, there appeared to be no constant relation between the color of an oil and its vitamin content. Thus, the mutton oleo oil, which was dead white in color, was richer in vitamin A than the light-yellow No. 1 oleo oil, and approximately as rich in this vitamin as the medium-yellow No. 2 and No. 3 oils.

As compared with the other commercial food fats and oils, oleo oil ranks below butter but ahead of lard and the vegetable fats and oils as a source of vitamin A.

LITERATURE CITED

(1) DRUMMOND, J. C., and COWARD, K. H.
1920. RESEARCHES ON THE FAT-SOLUBLE ACCESSORY SUBSTANCE. V. THE NUTRITIVE VALUE OF ANIMAL AND VEGETABLE OILS AND FATS CONSIDERED IN RELATION TO THEIR COLOUR. Biochem. Jour. 14: 668–677, illus.

(2) ——— and WATSON, A. F.
1922. THE TESTING OF FOODSTUFFS FOR VITAMINS. Analyst 47: 235–246, illus.

(3) HALLIBURTON, W. D., and DRUMMOND, J. C.
1917. THE NUTRITIVE VALUE OF MARGARINES AND BUTTER SUBSTITUTES WITH REFERENCE TO THEIR CONTENT OF THE FAT-SOLUBLE ACCESSORY GROWTH SUBSTANCE. Jour. Physiol. 51: 235–251, illus.

(4) OSBORNE, T. B., and MENDEL, L. B.
1915. FURTHER OBSERVATIONS OF THE INFLUENCE OF NATURAL FATS UPON GROWTH. Jour. Biol. Chem. 20: 379–390, illus.

(5) STEENBOCK, H., BOUTWELL, P. W., and KENT, H. E.
1918. FAT-SOLUBLE VITAMIN. I. Jour. Biol. Chem. 35: 517–526, illus.

(6) ——— SELL, M. T., and BUELL, M. V.
1921. FAT-SOLUBLE VITAMIN. VII. THE FAT-SOLUBLE VITAMIN AND YELLOW PIGMENTATION IN ANIMAL FATS WITH SOME OBSERVATIONS ON ITS STABILITY TO SAPONIFICATION. Jour. Biol. Chem. 47: 89–109, illus.

(7) UNITED STATES DEPARTMENT OF COMMERCE, BUREAU OF THE CENSUS.
1924–25. STATISTICS OF FATS AND OILS. (Preliminary report) 1924.

LIFE HISTORY OF THE VARIEGATED CUTWORM TACHINA FLY,[1] ARCHYTAS ANALIS[2]

By H. W. ALLEN

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INTRODUCTION

This species was described originally by Fabricius (*7, p. 311*)[4] in 1805 as *Tachina analis*. It was described as *Jurinia amethystina* by Macquart (*10, pl. 3, p. 199*) in 1842, as *Tachina apicifera* by Walker (*21, p. 718*) in 1849, and as *T. californiae* (*22, p. 270*) by Walker in 1856. During recent years several contributions to the distribution record have been made, most notable of which are those of Brimley (*5, p. 21*) and Johnson (*9, p. 206*), and it has several times been recorded as a valuable parasite of the variegated cutworm (*Lycophotia margaritosa* Haw.) and the army worm (*Cirphis unipuncta* Haw.). Sherman (*14, p. 301*) noted that this parasite emerges from the pupa of its host; and Reinhard (*13*) found that the adult is strongly attracted to flowers, and he listed the species to which it is attracted in Texas.

During the last two years the writer has been making a biological study of the tachinid parasites of Southern crop pests at the Mississippi Agricultural and Mechanical College. *Archytas analis* has been found to be one of the commonest and most beneficial tachinids in that section. It was discovered that very little concerning the details of its life history had been published, so a considerable part of 1924 was devoted to work with this insect. There are still many points in its biology which have not been definitely established, and others in which the data are rather limited. But considerable new information has been discovered, and since very few contributions to the biology of the group to which this species belongs have been made, the writer wishes to submit his results for publication at this time.

Biologically, this species belongs without question to Group IV of Pantel (*11, p. 32*) to which Baer (*3, p. 203*) has recently given the name Echinomyia group, and which Townsend has called the Hystriciine series (*17, p. 132*).

DESCRIPTION

The original description of the adult by Fabricius on specimens from South America, "America meridionale," is too superficial to be

[1] A common name suggested by its prevalence as a parasite of the variegated cutworm.

[2] Received for publication Aug. 25, 1925; issued February, 1926.

[3] The writer is indebted to W. C. Cook, J. J. Davis, C. L. Fluke, J. L. Hoerner, A. L. Melander, C. E. Mickel, H. C. Severin, M. H. Swenk, and R. L. Webster for contributions to the distribution record; to J. M. Beal, professor of botany, Mississippi Agricultural and Mechanical College, for determination of host plants; and to H. Gladney and J. K. Holloway for much assistance in the insectary and the laboratory.

[4] Reference is made by number (italic) to "Literature cited," p. 434.

of much present-day value in differentiating the species. However, several detailed descriptions of the species are available, and it can be readily determined by the keys in Coquillett's "Revision of the Tachinidae" (*6, p. 10, 142*). In order to facilitate a preliminary determination, the following diagnostic characters are given.

The adult is a moderately large, robust fly with a tawny yellow pollinose thorax, and a coal-black abdomen. The eyes are naked, ocellar bristles lacking; the third antennal joint is scarcely longer than the second, and is prominently convex on the anterior margin; the proboscis is nearly or quite as long as the height of the head, and the palpi are normal. It is distinguished from the several other closely related species of the same genus by the color of the abdomen and by the sides of the face being clothed with numerous fine white hairs (but lacking bristles) and by the color of the calypteres, which are white.

FIRST-INSTAR LARVA

Elongate oval when viewed from above; strongly compressed; submarginate at the sides, with a distinct groove extending longitudinally between the two lateral ridges. Intersegmentary constrictions well defined. Color grayish-white above, becoming white as the maggot grows. Young maggots (fig. 1) are

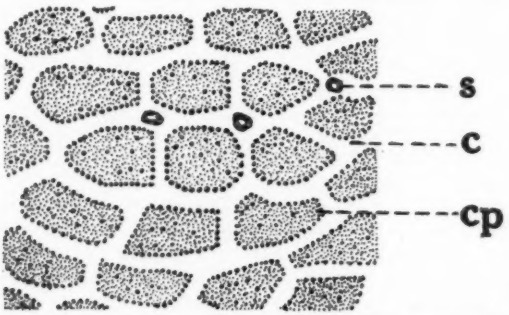


FIG. 1.—Section of the dorsal cuticula of a free living maggot, showing the thickened cuticular plates × 600; *c*, thinly chitinized space between the cuticular plates; *cp*, cuticular plate; *s*, sensorium

covered dorsally by a mosaic of coarse blackish polygonal cuticular thickenings, separated by fine white reticulations, in older maggots becoming separated by wide areas of white cuticula. Cuticular plates are evenly distributed over the dorsum, except at the intersegmentary constrictions, where they are lacking, and extend slightly ventrad of the lateral margin; under high magnification they are seen to be coarsely stippled. Venter of each segment with transverse band of minute serrate spines, not in serried rows. Buccopharyngeal apparatus (fig. 2) without articulations, median hook moderately stout, bluntly tipped, without denticles or dorsal serrations, and with delicate recurved hooks on either side of the extreme front. Dorsal wings of the basal part narrower than the sinus and about as wide as the ventral wing when viewed from the side; lower wing slightly longer than the upper wing. Metapneustic, posterior spiracle with two papillae; atrium (felt chamber) two to three times as long as its width, tracheal system scarcely visible through the cuticula. Free living maggots 0.55 mm. long, 0.13 mm. wide.

This description applies particularly to free living maggots on foliage, and to the early parasitic life in the subcuticular position. In the very much larger first-instar maggot found in the body cavity of the host, the cuticula becomes stretched through growth, the spines and cuticular plates widely separated, and the white delicate maggot is scarcely to be recognized as of the same instar as its free living counterpart.

SECOND-INSTAR LARVA

Triangular in outline when viewed from above, flattened ventrally, dorsum strongly arched, perceptible lateral prolongations in the pleural regions. Color white, cuticula transparent. Small, but well defined black spines in long transverse rows on the anterior half of the ventral part of the thoracic segments; last four segments with extremely minute brown spines in long transverse rows on posterior third or more of the segment, extending ventrally except on the anterior two of the four; elsewhere without spinyarmature. Mouth parts with two articulations; hooks bifid, the two prongs parallel and separated by a deep, slitlike cleft, each provided with a strong rounded ventral denticle and an equal dorsal prolongation ending in a strong point. In profile view, the dorsal wings larger then the ventral, from which they are separated by a sinus scarcely one-third the width of the dorsal wing; basal piece evenly and heavily chitinized to posterior margin. Metapneustic; each posterior spiracle separated by three times width of one; spiracle with two slits, the sides of which are almost parallel to the horizontal plane.

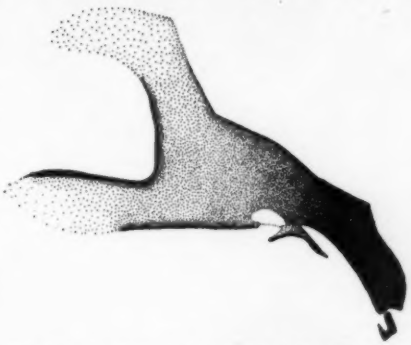


FIG. 2.—Buccopharyngeal apparatus of the first-instar maggot. × 400

The second-instar maggot occurs in a highly characteristic hump of the wing pad of the living pupa of its host, through the semitransparent walls of which it may often be clearly seen.

THIRD-INSTAR LARVA

Somewhat compressed; triangular in outline when viewed from above (pl. 1, F). Color white, cuticula thin, delicate, transparent. Cuticular aramature scanty, consisting of several rows of minute black spines on the anterior part of the thoracic segments, similar spines in undulating rows on the whole surface of the last segment, dorsum and venter of the penultimate and antepenultimate, and venter of the intermediate abdominal segments. In the pleural region there is a very distinct series of three rows of fleshy callosities extending the length of the body. Buccopharyngeal apparatus (fig. 3, A) resembles that of the second instar, excepting that there are three articulations, and the mouth hooks are more bluntly pointed. Amphipneustic; anterior spiracle (fig. 3, D) consisting of two to three small black parallel slits or papillae not protruding conspicuously above the surface of the surrounding cuticula; posterior spiracles separated from each other by less than the width of one (fig. 3, C). Spiracle with three nearly straight slits on smooth, black, elevated ridges (fig. 3, B) converging on a black button located near the middle of the inside margin.

The large third-instar maggot occurs in the dead pupa of its host, the cavity of which is almost completely filled with its soft white body.

Greene's description of the puparium (*8, p. 26*) is as follows (fig. 4):

Large; dull, dark red, with a faint indication of a depression or stricture at the posterior end. Spiracles shining black, slightly raised above the surface, separated by a space nearly equal to the width of one plate. Each plate has three slits, each located at the top of a well-defined ridge. Button fairly large, round. Spiracles above longitudinal axis, about two-thirds the width of one plate. Anal opening very small, far below the spiracles. Length, 10.5 mm.; diameter, 4.75 mm.

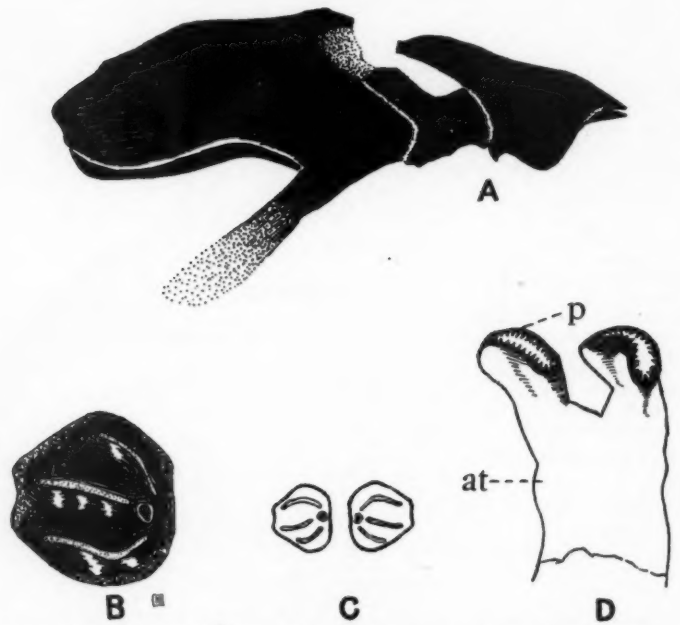


FIG. 3.—Details of structure of third-instar larva. A, buccopharyngeal apparatus, × 40; B, posterior spiracle, × 20; C, outline of both spiracles, showing their relative position, × 8; D, anterior spiracle, lateral aspect, × 96; *at*, atrium; *p*. papilla

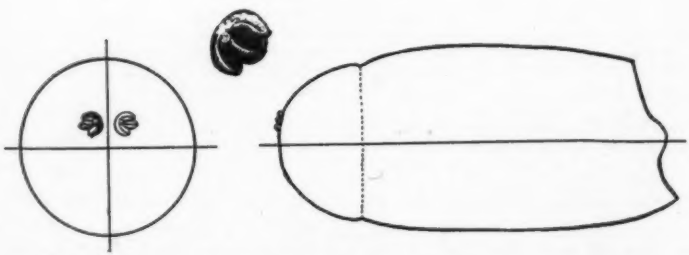
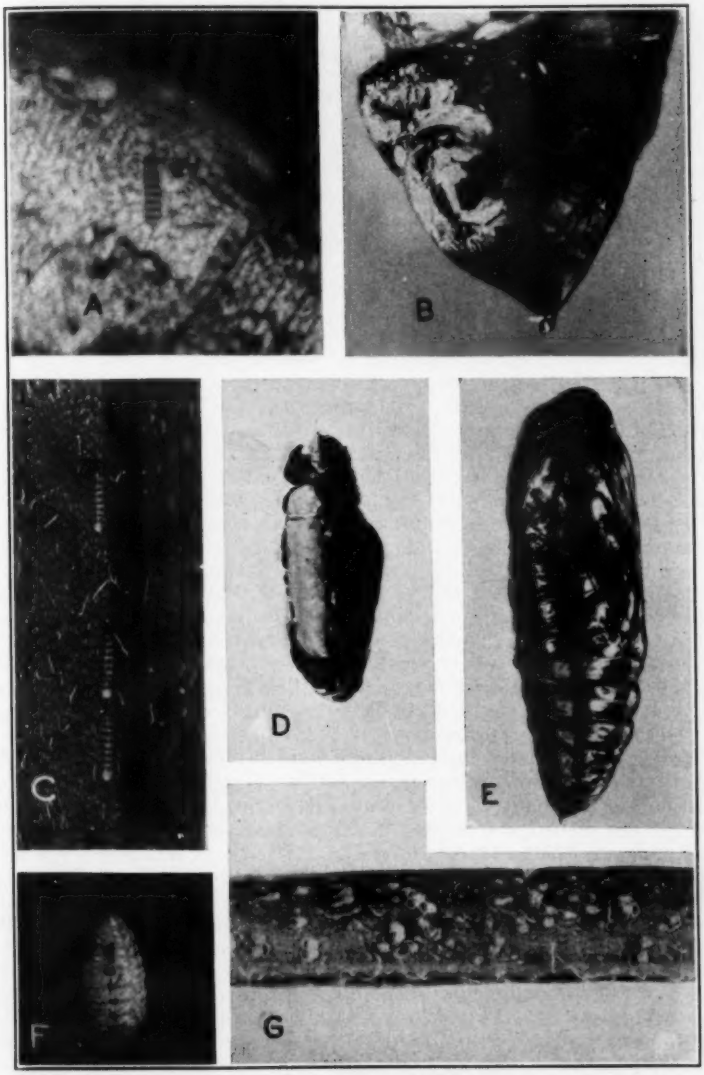


FIG. 4.—Details of the structure of the puparium, showing the outline when viewed from the side, the location of the posterior spiracle when viewed from behind, and the detailed structure of the posterior spiracle. (After Green)



A.—First-instar maggot in subcuticular position in variegated cutworm. × 25
B.—Posterior end of pupal case, showing characteristic breathing slit torn by the third-instar maggot of *Archytas analis*. × 25
C.—Free living first-instar maggots seated in cuplike attachment, and lying closely pressed to the substratum. × 25
D.—Puparium in position in pupal case of host, the upper half of which has been removed to show position of puparium. × 3
E.—Pupa of the variegated cutworm, showing the characteristic swelling of the wing-pad produced by the presence of the second-instar maggot of *Archytas analis*. × 5
F.—Third-instar maggot, dorsal view. × 2.5
G.—Membranous attachments of free living maggots remaining on the substratum after the maggots have disappeared. × 30

LIFE CYCLE

The life cycle of this species was worked out under out-of-door insectary conditions. Adults were maintained very satisfactorily in wooden cages with a base 6 by 10 inches and a depth of 4 inches. The bottom of the cage was kept covered by moist earth, and over the top was placed a tightly fitting glass plate. In one end of the cage a 1-inch hole plugged with cotton served partly for ventilation, but particularly for added convenience in manipulation. Food and water were provided and changed daily. In order to facilitate the daily manipulations, a special cage was built inside the insectary, just large enough to give working space to one worker with a breeding cage. One wall facing the light was screened. All the other walls were covered with black cloth to shut off the light. It was found that flies leaving the cages, when food and water were being changed, would almost invariably remain on the outside screen, responding to their strongly positive phototropic instinct, and were readily picked up in small vials and returned to their proper cages when ready to receive them once more. Sugar water, diluted honey, and mashed banana spread on small pieces of cardboard were furnished for food, and whenever possible sprigs of melilotus bloom, or other favored host-plant flowers were placed in the cage with the stems in water, and maintained with the other food. Water was furnished from saturated sphagnum moss in 1-ounce salve boxes. Larviposition was readily obtained on herbaceous foliage in the insectary. From such stock, transfers of maggots from foliage to caterpillar were readily accomplished by laboratory manipulation. Infested caterpillars were thereafter maintained in 1-ounce tin salve boxes, jelly glasses partly filled with soil, or under inverted jelly glasses placed over flower pots filled with soil. Puparia were maintained most successfully in sphagnum moss just wet enough to feel moist to the touch.

Adults mate almost immediately after the emergence of the female. Mating is followed by the preovispositional period during which time the ova in the body of the female are fertilized. During the same period the fertilized ova pass down the long, more or less quadrate uterovagina, where they develop into active maggots inclosed in a thin chorion sheath. Females, after reaching maturity, desposit minute maggots in enormous numbers on the stems, leaves, and flowers of the hosts' food plants. The maggot lies more or less quiescent on the substratum upon which it was deposited, until a suitable host caterpillar passes within reach, when the maggot attaches itself to the body of the caterpillar and crawls to some protected place where it again becomes quiescent. It then bores through the integument, a process requiring several hours, penetrating obliquely, and taking a position between the hypodermal layer and the cuticula. After spending several days in this position, the maggot penetrates the body cavity, in which it passes over into the pupal stage of the host. Here it occurs first in a sort of cradle in the bulging wing-pad, with a breathing pore to the outside. The death of the pupa soon takes place. In its rapidly putrifying contents the maggot completes its development and invariably pupates within the pupal case of its host, from which the fly emerges a few days later.

DURATION OF THE LIFE STAGES

The durations of the various life stages of analis have been summarized in Table I which may require a few words of explanation and qualification.

TABLE I.—*Duration of the life stages of Archytas analis*

Life stage	Time of experiments	Number in experiment	Duration in days		
			Minimum	Maximum	Average
Adult:					
Preovipositional	Middle of June to middle of August	9	8	19	10.6
Larvipositional	Middle of June to late August	7	3	30	13.3
Longevity—					
Males	Late June to early September	16	2	53	19.9
Females	do	20	1	47	23.9
Both sexes	do	36	1	53	22.0
Larva:					
Free living—					
Lot 1	Late April to early June	31	2	31	18.1
Lot 2	July	*90	4	16	.....
Parasitic—					
Lot 1	May	1	24	24	24.0
Lot 2	Late June to late July	23	7	18	11.6
Lot 3	Late August to middle of September	3	13	18	16.0
Puparium:					
Lot 1—					
Males	Late May to middle of June	9	10	13	11.0
Females	do	6	11	12	11.8
Both sexes	do	15	10	13	11.4
Lot 2—					
Males	July	9	10	11	10.4
Females	do	12	11	13	11.6
Both sexes	do	21	10	13	11.1
Lot 3—					
Males	September	2	13	13	13.0
Females	do	2	14	14	14.0
Both sexes	do	4	13	14	13.5
Combined parasitic larva and puparium:					
Lot 1	Late June to early August	23	19	30	22.6
Lot 2	August	4	21	23	22.0
Lot 3	Late August and September	4	25	31	28.5

*Approximate.

The prelarvipositional and also the larvipositional period was determined for females copulating on the day of emergence, fed and watered daily, and allowed to run with males until the death of the latter. During the larvipositional period maggots were not deposited every day, 1 to 3 days frequently elapsing without larviposition. Death of the female was customarily preceded by 1 to 5 days of reproductive inactivity; in one case, after 5 days of normal larviposition, by 23 days of inactivity. Three-fourths of the maggots obtained in this experiment were deposited in from 10 to 20 days following the emergence of the female. Adult longevity was determined from specimens maintained in the insectary in the normal manner throughout the hotter summer months.

The free living first-instar maggots are remarkably hardy. Their longevity was found to vary considerably with the season, being longer in the cooler months. In lot 1, individuals attached to short

sections of melilotus stems were maintained in the insectary in covered jelly glasses, under somewhat unfavorable conditions. In lot 2, about 90 maggots were deposited on July 1, on a small potted melilotus plant, which was maintained in a vigorous growing condition throughout the experiment, and upon which the surviving maggots were counted each day. Maggots began to die on the fourth day, the death rate remaining very low until the eighth day, then increasing rapidly until the death of the last one on the sixteenth day. Maggots deposited on sprigs of Amaranthus and chrysanthemum, in the late fall, passed the short, mild winter successfully, the majority remaining vigorous for more than a month, some surviving until February 25, with a longevity of at least 52 days. At the end of this long period, the substratum had become incrusted with mold, more or less desiccated, and to all appearance highly unfavorable to the health of the attached maggots. In all probability, maggots deposited at the same time on the foliage of hardy plants in the field came through the winter in much better condition. The longevity of the free living maggots is not an exact measure of the period during which they are infective. Maggots on the substratum are often so weak for several days before they perish that they are unable to effectively establish themselves on a passing host. It was found, however, that the maggots, deposited and maintained on potted melilotus under conditions approximating the normal, were able to establish themselves readily and to penetrate the body of the host up to 14 days after deposition, even during the most unfavorable midsummer weather. The effective longevity of free living maggots during the cooler months was not determined, but is undoubtedly much longer.

The maggot duration on and in the host is highly variable, depending directly on the time required for the host to attain its pupal stage, and indirectly on the instar of the host infested, the degree of divergence from optimum temperature for the host, and other factors. Durations under the influence of variable temperatures are indicated in the three lots in Table I. The host for lot 1 was the variegated cutworm; for lot 2, the yellow-striped army worm (*Prodenia orno-thogalli* Guen), in the last three instars; and for lot 3, *Cirphis juncicola* Guen, in the last two instars. The pupal period was determined at three different times, and it also was found to vary with the season, but not to such a marked degree as the parasitic larval stage. The combined maggot and pupal duration from the time of infestation of host to the emergence of the adult fly was determined for three different lots, and constitutes a more precise determination of the duration of this period than that obtained by combining the separate data of larval and pupal durations. Lots 1 and 2 were reared from the yellow-striped army worm, lot 3, from *Cirphis juncicola*.

The duration of the life cycle during the active developmental season was found to be as follows: Prelarvipositional adult life 8 to 19 days, larvipositional life up to 30 days, free maggot life 0 to 31 days, parasitic maggot life 7 to 24 days, puparium 10 to 14 days, with a minimum of 25 days and a maximum of 118 days for the completion of the cycle. The overwintering generation almost certainly has an even longer cycle, but its duration has not been determined.

SEASONAL CYCLE

The seasonal cycle has not been fully worked out. In north central Mississippi there seem to be two complete broods each year. The earliest-appearing individuals of each of these broods pass through to maturity in a comparatively short time, giving rise to several partial broods. But the life cycle of the late-appearing individuals of each brood is much extended, producing broadly overlapping generations from very early in the season. In the insectary during the season of 1924 the beginnings of five distinct broods were obtained, and in the field during the same period there may have been one or two more.

The manner in which *Archytas analis* hibernates has not been definitely determined. Free living maggots on substratum succeeding in passing the winter successfully in the insectary, some surviving as late as February 25, after withstanding more than one exposure to freezing temperature and a very long inactive period on the substratum. All died, however, in the early spring before suitable caterpillar hosts could be provided, and it appears to be by no means certain that in the field the winter is successfully passed in this stage.

HABITS OF THE ADULT

FEEDING

Reinhard (*13*) has collected adults of analis from broomweed (*Amphiacyris dracunculoides*), bitterweed (*Helenum tenuifolium*), wild aster (*Aster lateriflorus*), *Rudbeckia bicolor*, sweet clover (*Melilotus alba*), and prairie cacia (*Cecuan illinoensis*) in Texas. Townsend (*16, p. 177*) has taken them from flowers of *Lippia lanceolata* in the same State. Aldrich (*2, p. 83*) records taking them from sweet clover and golden rod.

In common with other tachinids possessing an elongate proboscis, this species is preeminently a flower-visiting, nectar-feeding species. Several flies collected on melilotus have been dissected and the crops examined. In all cases the crops were distended with a clear fluid, not mixed with pollen grains or other solid substances, and giving a strong positive reaction to Fehling's solution, indicating the presence of glucose. In Mississippi the adults are most commonly attracted to the flowers of herbaceous plants in open fields, they having been frequently observed in conspicuous swarms at the flowers of sweet clover, hairy vetch, smartweed (*Polygonum pennsylvanicum L.*), chrysanthemum, and at the flowers and nectaries of smooth vetch. They have also been noted less frequently at the flowers of crimson clover, *Chaerophyllum Teinturieri* Hook, *Geranium carolinianum* L., the wild sweet pea (*Lathyrus pusillus* Ell.), *Bidens aristosa* Brit., *Aster ericoides pilosus* Port., and soy bean. While they are usually attracted to herbaceous plants, shrubs or even tree tops are visited in search of food, a few adults having been observed feeding from the flowers of *Spirea vanhoutei*, and at the nectaries on the leaf petioles of peach. While nectar is unquestionably the principal food of the adult, honeydew is sometimes taken, adults having been observed feeding on honeydew-smeared foliage of tulip trees (*Liriodendron tulipifera* L.) beneath a heavy infestation of the tulip tree soft scale

(*Toumeyella liriodendri* Gmel), and on the foliage of turnips covered with the honeydew of the turnip aphis (*Rhopolosiphum pseudobrassicae* Davis). In confinement, adults feed very freely on sugar, diluted honey, mashed banana, and similar sweets.

COPULATION

Copulation has never been observed in the field, but in insectary cages there was plenty of opportunity to observe it between females only a few hours from the puparium and males a day or more old. The sexes remain in coitu from four to five minutes. Fertilization of the female in the field undoubtedly takes place almost as soon as its wings have expanded.

LARVIPOSITION

Deposition of larvæ has been noted occasionally in the field, and many times in the insectary. As the female walks over the stems, she frequently touches the substratum with the tip of her abdomen, depositing thereon a maggot. Covering the posterior end of the maggots being deposited is a minute membranous cup. At the time of deposition the female fastens this cup securely to the substratum, furnishing the maggot with a fixed base but leaving the remainder of its body free. At the height of its larvipositional period the female may deposit as many as 10 to 12 maggots per minute. Periods of deposition alternate with periods of rest. With flies confined in cages it was found that on those plants having both stems and leaves a large majority of the maggots were deposited upon the stems, but some were also deposited on the leaf surface, stipules, blossoms, in fact, any succulent exposed part of the plant. When deposited upon leaf blades, the under side was preferred. Flies in confinement larviposited quite freely on the foliage of several species of herbaceous plants, none being avoided. Prell (*12*) has advanced the idea that adult tachinids are attracted to infestations by a more or less diffuse infestation odor, but that these flies, when once present in an infestation, utilize something other than the sense of smell to locate the individual parasite. Such a diffused infestation odor was undoubtedly present under insectary conditions, but larviposition was at all times readily obtained in cages without the stimulation of the actual presence in the cage of host caterpillars. Maggots are usually deposited with their axes parallel to that of the stem or leaf upon which they are placed.

SEGREGATION OF SEXES IN THE FIELD

Adults were collected at frequent intervals during the summer of 1924. The proportion of the two sexes bore no fixed relation to the normal, and indicated sexual segregation in the field, as may be noted in the following tabulation:

TABLE II.—*Showing periodical fluctuation in proportion of sexes in the field*

Date	Males	Females	Where collected	Date	Males	Females	Where collected
Apr. 15	0	2	Flowers of crimson clover.	Aug. 18	0	1	Flowers of soy bean.
Apr. 21	0	3	Smooth vetch.	Aug. 19	0	2	About colony of fall army worm.
May 15	0	1	On wild sweet pea.	Do.	0	4	At nectaries of cowpea.
May 19	3	0	Nectaries of peach.	Aug. 23	0	1	About colony of fall army worm.
June 10	1	1	Flower of melilotus.	Aug. 24	0	1	At nectaries of cowpea.
June 17	4	0	Foliage under pecan trees.	Sept. 10	8	1	Over ground previously infested with fall army worm.
Do.	15	0	Grass under apple orchard.				
June 20	8	2	Flowers of melilotus.				
June 23	9	1	Do.				
July 6	2	3	Do.				
July 11	3	0	Do.	Do.	1	1	Flowers of Bidens.
July 26	1	0	Do.	Sept. 15	1	1	Over Johnson grass.

A study of the above grouping will show that in regard to the proportion of sexes, four rough seasonal divisions can be made. The first, from April 15 to May 15, in which only females were encountered, coincides roughly with the period of local infestation of the variegated cutworm when the caterpillars were abundant. The second, from May 19 to July 26, represents the main swarming period for analis for the season, when adults were present in enormous numbers at the flowers of melilotus. During this period both sexes were present, but males were several times more numerous than the females. This period was contemporaneous with the emergence of analis and the surviving host adults from the heavy local infestation of variegated cutworm. Reestablishment of the infestation failed to occur, and no suitable hosts were at that time locally abundant. The third period, from August 18 to August 24, was again marked by the presence of numerous females, and practically no males. This period coincided with heavy outbreaks of the fall army worm (*Laphygma frugiperda* Guen) mixed with *Cirphis juncicola*, about which the adult flies were abundant. The fourth period, from September 10 to September 15, was marked by the presence of many males and only a very few females, similar to the second period. It coincided with the emergence period of parasites and host adults from the colonies of the fall army worm and *Cirphis juncicola*. Here again the hosts failed to reestablish the infestation, practically vanishing from the locality, and no caterpillars suitable for parasite attack could be found in the vicinity.

The underlying reasons for this segregation, from the limited nature of the data, are highly conjectural. The preponderance of males at one time and females at another can not be explained by differences in proportion of sexes, which were always nearly equally divided in insectary rearings; nor by differing times of emergence, for in all insectary work, the males come out only a day or two at the most before the females; nor by the early death of the males, for their longevity, as determined in the insectary, is only slightly less than that of the female. Adults of analis are powerful in flight, and it seems quite likely that females, after being fertilized, if suitable hosts are not locally abundant, migrate out of the region in which they have passed their parasitic existence, leaving the males behind to mate with the other females as they emerge from day to day.

It is certain that the seasonal fluctuations in abundance of adults in the field, which are so conspicuous, should in no way be interpreted as the flight periods of well differentiated broods, but rather as variations in the status of the parasite due to the constantly changing conditions in the abundance of foods and favored hosts.

ACTIVITY IN RELATION TO METEOROLOGICAL CONDITIONS

Adults of analis are active in the field under wide extremes of atmospheric conditions. In midsummer they frequently have been observed displaying normal activity in dry open fields, at midday with the temperature as high as 94° F. and the relative humidity from 52 to 59 per cent. The normal hosts are not usually found in abundance under such extreme conditions of heat and dryness. These observations indicate that the adult parasite can withstand any degree of heat and dryness not inhibitive to the host. At the other extreme, it has been observed that adults may be found in the cold, early winter days, when nearly all other adult tachinids have disappeared. Thus adults of analis have been taken flying about flowers on a chilly, cloudy day with the temperature at 67° F. In insectary experiments, adults were inactive at temperatures below 63° F.; from 63° to 73° their activity was limited to walking, running, and sluggish flying; at temperatures above 73° they were very active.

HABITS OF THE LARVAE

FIRST INSTAR

So long as they are not disturbed, the free living maggots remain seated in the basal cuplike membrane, as deposited by the female, with the body lying quiescent and closely pressed to the substratum (pl. 1, C), in a position in which they are manifestly protected from the rigors of adverse atmospheric conditions by the dorsal covering of thickened cuticular plates. As Prell has indicated in the nearly related *Panzeria rudis* (*12*), further protection is afforded by the basal membrane, which partially seals the only functional spiracle possessed in this stage. Whenever the substratum is disturbed, the maggot rears upwards (fig. 5) on its posterior end, swinging the head about in wide circles in an attempt to touch its host. Frequently, in its excess of excitement, a maggot will deliberately crawl away from its basal attachment to the substratum in order to bring itself within striking distance of its host, whose presence it has perceived. A maggot failing to make a suitable contact subsides to the original position closely pressed to the substratum. If, however, it strikes the body of a passing host it immediately releases itself from the basal membrane, which remains attached to the substratum (pl. 1, G), and crawls onto the body of the host. Maggots attach themselves readily to at least the last three larval instars. Maggots on substratum exhibit some powers of differentiation of possible hosts, but this power is manifestly not very highly developed. For instance, maggots attach themselves quite readily to caterpillars of the variegated cutworm, the army worm, the fall army worm, all of which serve as normal hosts, as well as the yellow-striped army worm and *Cirphis juncicola*, from which they have been reared after artificial infestation. But they will also attach themselves readily to the beet army worm (*Laphygma exigua* Hueb.), the grape leaf-folder (*Desmia*

funeralis Hueb.), the garden webworm (*Loxostege similalis* Guen.), and the dingy cutworm (*Feltia subgothica* Haw.). Into two of the latter, namely the beet army worm and the dingy cutworm, the maggots were able to penetrate in a normal manner, but they failed to complete their development, and there is no record that any of these ever serve as normal hosts. On the other hand, some caterpillars are deliberately refused. Maggots brought into contact with the caterpillars of the corn-ear worm (*Heliothis obsoleta* Hueb.), the granulate cutworm (*Feltia annexa* Treit.), the cabbage looper (*Autographa brassicae* Riley), and the bagworm *Thyridopteryx ephemeraeformis* Haw.) persistently refuse to attach themselves. None of these caterpillars are normal hosts.

After attaching itself to the body of its host the maggot crawls about for a few minutes, usually coming to rest in some more or less protected fold of the cuticula. Among 257 maggots allowed to establish themselves on host caterpillers confined with them in breeding cages, it was found that the venter and pleuron of the host body was preferred to the dorsum, and that many more sought out the first five segments than the other segments of the body, though maggots were generally scattered over all parts of the body, except the heavily chitinized head. The maggot exudes a liquid substance which on drying fastens its body securely to the cuticula of the host, where it may remain quiescent for 24 hours or more. It finally cuts a hole in the cuticula, and slowly works its way into the body, entering at an extremely oblique angle, so that it lies just beneath the cuticular and between it and the hypodermal layer, where it can be distinctly seen from the outside in caterpillars having a thin cuticula (pl. 1, A). Penetration is effected without leaving a scar, and no surface breathing pore with the attached breathing funnel is maintained. Subcuticular maggots, while still in the first instar, and while the host is still an active caterpillar, bore through into the body cavity, where they appear to wander freely about until the end of the feeding period of their host. At this time the maggot takes up a fixed position in the thoracic region, molting there to the second instar as the host is transforming to the pupal stage. Many maggots may penetrate the body of the host, but rapid reduction in numbers takes place among those which penetrate the body cavity, so that when the second instar is reached only two or three remain alive.

FIG. 5.—Free living, first-instar maggot, in characteristic erect position, seated in its cuplike attachment to the substratum. ×50

SECOND INSTAR

The presence of the young second-instar maggot in the newly formed pupa induces the production of a delicate membranous sheath, which is somewhat more heavily constructed toward the wall of the host. The maggot within this sheath is found in the newly formed pupa cradled in a highly characteristic bulge of the wing pad (pl. 1, E). No breathing funnel is developed, but the posterior

spiracles of the maggot are applied to a rupture at the lower end of the wing pad, through which air is obtained. After pupation of the host, maggot development is much accelerated, the maggot growing rapidly, killing its host at the end of the second or the beginning of the third instar.

THIRD INSTAR

At the death of the pupa the third-instar maggot grows very rapidly, living largely on decaying tissues, all of the softer parts being consumed, the maggot becoming so large as nearly to fill the interior of the host pupa with its bulk. In this instar the maggot obtains its necessary supply of air by cutting a minute hole in the extreme front of the pupal case, to which the anal spiracles are applied as it works toward the posterior end of the pupa. When feeding is nearly completed, several much larger slits are torn about the periphery of one of the segments near the anal end (pl. 1, B), so that the latter is frequently nearly severed. The maggot then faces about so that its anterior end lies in the anterior end of the pupal case, and pupates, the puparium completely filling the middle part of the host pupal case (pl. 1, D).

RELATION OF THE PARASITE TO ITS HOST

The host caterpillar displays no marked irritation when the maggots attach themselves to its body, crawl over the surface, or even when they penetrate the cuticula. Unless heavily overstocked with maggots, it completes its development to the pupal stage in a normal manner, the presence of several maggots in their first instar producing neither abnormal symptoms nor appreciable lesions in their host. In the variegated cutworm, pupation normally occurs in an earthen cell an inch or more beneath the soil surface. During its early pupal life in this cell the host succumbs to the parasite, which completes its development on the disintegrated contents of its host, transforming to a puparium in its empty pupal case.

Rather early in the season a number of variegated cutworms were dissected to study the development of the parasite within its host. In this lot it was found that first-instar maggots occur in the subcuticular position up to about 15 days from time of penetration, second-instar maggots were encountered from the sixteenth to the twenty-second day, and the third instar from the twenty-second to the twenty-sixth day.

The speed of development of the parasite, which must necessarily await the pupal stage of the host before being enabled to complete its parasitic existence, is largely dependent on the rate of development of its host. This is shown very clearly in Table III. Those caterpillars infested in their last instar allowed the parasite to complete its development to the adult in 20 days and those infested in the penultimate instar in 22.2 days.

TABLE III.—*Difference in speed of development of maggot attacking different instars of the host*

Instar of host when infested	Number in experiment	Number of days from—						
		Infesting to host, a prepupa	Host, a prepupa, to host a pupa	Host, a pupa, to pupal death	Pupal death to formation of puparium	Total maggot duration	Formation of puparium to fly emergence	Total maggot and pupal duration
Last	10	4.0	1.5	1.6	1.5	8.6	11.6	20.0
Penultimate	6	4.5	2.2	2.0	2.6	11.3	10.9	22.2
Antepenultimate	6	9.1	1.8	2.1	1.8	14.8	10.6	25.4
Averages		5.9	1.8	1.9	2.0	11.6	11.0	22.6

The antepenultimate-instar caterpillars, which required considerably longer time to attain the pupal stage allowed their parasites to emerge only after 25.4 days. It was also determined that the rate of development of the parasitic life was not appreciably influenced by the duration of the preceding free living maggot stage, whether the latter was of only a few hours duration or a matter of days.

FECUNDITY

The reproductive power of *Archytas analis*, as with other parasites depositing their progeny on the food plant of the host, is very high. The largest number of maggots deposited by an insectary-reared female was 579, and by any captured female, 531. The average number produced by insectary-reared females was 270, and the numbers deposited daily ranged from 0 to 290. These numbers are very probably less than in the field under more favorable conditions. One laboratory-reared female, dissected before larviposition began contained 890 maggots and undeveloped ova. This great reproductive power is largely counterbalanced by the high percentage of ineffectives among the free living maggots, comparatively few of which are ever confronted with an opportunity to establish themselves on a host.

DISTRIBUTION

Archytas analis is generally distributed throughout the United States (fig. 6), where it has been reported from all sections, excepting the extreme Southwest. It also occurs in southeastern Canada (*1*, *p. 485*), Santo Domingo, Jamaica, St. Vincent, Colombia, and Venezuela. It has not been reported outside of the Americas. While it appears that this parasite is present only in the Western Hemisphere, it has, within the limits of its range, become adjusted to a very wide variation of climatic conditions, being present from the north temperate to well into the tropical zone, and in the United States from the semi-arid Southwest to the humid forest region of the East and far West.

HOSTS

This species is moderately polyphagous, so far as known, attacking only caterpillars. It has been reared from seven different hosts represented by two families, the Noctuidae and Lasiocampidae. The variegated cutworm is undoubtedly its principal host throughout most of its range. It is also of considerable importance as a parasite of the army worm. Vickery (*18, p. 391*), records it as a parasite of the two nearly related species, *Cirphis latiuscula* H. and S. and *C. multilinea* Walk. In Colorado it has been reared from 2 species of tent caterpillars, *Malacosoma californica* Pack (*15, p. 70*) and *M. fragilis*

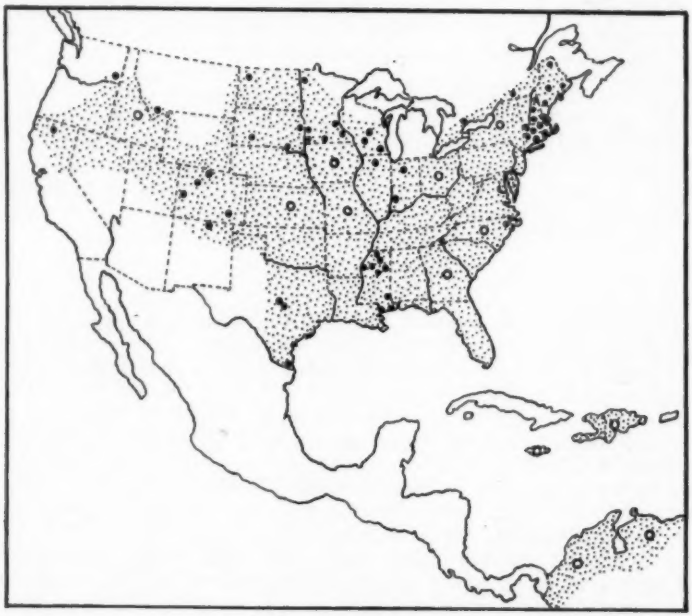


FIG. 6.—Map showing distribution of *Archytas analis*. Large dots represent locality records. Circles represent state or national records

St. (*4, p. 174*). In Mississippi it has been reared from the variegated cutworm and the fall army worm. It has been inoculated upon and reared quite successfully upon the army worm, *Cirphis juncicola*, and the yellow-striped army worm. Though the latter proved to be an excellent host for insectary work, analis, strange to say, has never been reared from material of this species collected in the field.

ECONOMIC IMPORTANCE

During the spring of 1924, in Mississippi, this species was reared from 14.9 per cent of the variegated cutworms captured during the last three instars. Last-instar caterpillars were most heavily parasitized, in one lot 36 per cent being destroyed by this parasite. During 1925, 22 per cent were parasitized. These percentages are

undoubtedly somewhat less than the total proportion of the infestation destroyed by analis, for while the earlier instars are readily attacked, the last-instar caterpillars are naturally much more liable to be parasitized, since they have lived a longer time exposed to infestation. *Archytas analis* has also been cited as a natural control of the variegated cutworm in Colorado (*20, p. 18*), and Wadley (*19, p. 276*) states that this pest was heavily parasited by it in Kansas in 1915 and in Iowa in 1919. Though it has been mentioned from several localities as a parasite of the army worm, no precise information is available as to its effectiveness on this host. During the last two seasons, at the Mississippi Agricultural and Mechanical College, with moderate numbers of army worm present, analis parasitism has been negligible. The writer was surprised at obtaining the parasite from the fall army worm; however, the record is unquestionable, since determination of the host was checked by examination of the pupal case after the fly had emerged. Its occurrence in the fall army worm is not common, and as a natural control of this pest is not important. It has been reported as a parasite of tent caterpillars, but its true value as a natural control for these pests has not been determined.

The possibilities for manipulation of this parasite, for the purpose of increasing its effectiveness, are much greater than for most tachinids. It displays great fondness for the nectar of certain food plants. Some of these, fortunately, are valuable legumes, the cultivation of which would not only serve to induce beneficial concentrations of these parasites, but they have an important place in improved farming methods. Melilotus and the vetches are particularly favored by analis adults. Certain other plants, such as *Polygonum pennsylvanicum*, which grow in great profusion on waste land and attract great numbers of the adult flies of this species, and which are regarded as weeds, might often be left standing with profit until after the blooming period is past.

It was found that free living maggots on stems of melilotus, the bases of which were wrapped in a ball of moist sphagnum moss, were able to withstand a five-day trip by mail, even in hot midsummer weather, coming through with a fairly high percentage of active living maggots. Such material could be readily obtained, shipped several hundred miles, and colonized. Free living maggots packed in sphagnum moss did not, however, respond favorably to retention in cold storage, none surviving a 30-day exposure to temperature varying from 37° to 40°.

The ease with which this species can be reared in confinement, and infestation of its hosts obtained, together with its enormous powers of reproduction, combined with the fact that it is an effective parasite of at least two highly important crop pests which are not always controlled by artificial methods, makes it a highly promising subject for experimentation in the field of biological control.

SUMMARY

Investigations were made upon the life history of *Archytas analis*, at the Mississippi Agricultural and Mechanical College in 1924. The parasite was found to be one of the group which deposits maggots on the foliage frequented by the host. These free living maggots,

which may live on the substratum for many days, attach themselves to passing caterpillars, if they are acceptable hosts, and bore through the cuticula to a position between it and the hypodermal layer, later passing into the body cavity, where they persist in the host until its pupal stage. The puparium is formed in the pupa of the host, only one parasite completing its development in each pupa. The time durations of the different stages differ widely with the season, the instar of the host when infested, and the time spent as a free living larva. There appear to be at least two complete and several partial broods each season. Adults are preeminently nectar suckers, and congregate in swarms at the flowers of sweet clover and a number of other common wild and cultivated plants. They are powerful flyers and there is some reason to believe that they migrate rather freely. They are present from early spring to late fall and are adapted to activity in wide extremes of weather. The parasite is broadly distributed over North America and parts of South America. It is moderately polyphagous, attacking several species of Lepidoptera. It is undoubtedly a valuable natural control for the variegated cutworm, and is also of some value as a parasite of several other caterpillar pests. It offers more promise than some species as a possibility in the field of biological control.

LITERATURE CITED

(1) ALDRICH, J. M.
1905. A CATALOGUE OF NORTH AMERICAN DIPTERA. 680 p. City of Washington.

(2) ———
1915. RESULTS OF TWENTY-FIVE YEARS' COLLECTING IN THE TACHINIDÆ. Ann. Ent. Soc. Amer. 8: 79–84.

(3) BAER, W.
1920. DIE TACHINEN ALS SCHMAROTZER DER SCHÄDLICHEN INSEKTEN. Ztschr. Angew. Ent. 6: 185–246.

(4) BAKER, C. F.
1895. BIOLOGICAL NOTES ON SOME COLORADO DIPTERA. Ent. News 6: 173–174.

(5) BRIMLEY, C. S.
1922. LIST OF THE TACHINIDÆ (DIPTERA) OF NORTH CAROLINA. Ent. News 33: 20–26.

(6) COQUILLETT, D. W.
1897. REVISION OF THE TACHINIDAE OF AMERICA NORTH OF MEXICO. U. S. Dept. Agr., Div. Ent. Tech. Bul. 7, 156 p.

(7) FABRICIUS, J. C.
1805. SYSTEMA ANTLIATORUM. 372 p. Brunsvigae.

(8) GREENE, C. T.
1922. AN ILLUSTRATED SYNOPSIS OF THE PUPARIA OF 100 MUSCOID FLIES (DIPTERA). Proc. U. S. Natl. Mus., v. 60, no. 2405, 39 p., illus.

(9) JOHNSON, C. W.
1925. FAUNA OF NEW ENGLAND. 15. LIST OF THE DIPTERA OR TWO-WINGED FLIES. Occas. Papers Boston Soc. Nat. Hist., v. 7.

(10) MACQUART, J.
1840–[43]. DIPTÈRES EXOTIQUES. t. 2, illus. Paris.

(11) PANTEL, J.
1910. RECHERCHES SUR LES DIPTÈRES À LARVES ENTOMOBIES. I. CARACTÈRES PARASITIQUES AUX POINTS DE VUE BIOLOGIQUE, ÉTHOLOGIQUE ET HISTOLOGIQUE. Cellule 26: 27–216, illus.

(12) PRELL, H.
1915. ZUR BIOLOGIE DER TACHINEN PARASETIGENA SEGREGATA RDI. UND PANZERIA RUDIS FALL. Ztschr. Angew. Ent. 2: 57–148, illus.

(13) REINHARD, H. J.
1919. PRELIMINARY NOTES ON TEXAS TACHINIDAE (DIPTERA). Ent. News 30: 279–285.
(14) SHERMAN, F., JR.
1915. REARING OF MOTHS AND TACHINA-FLIES FROM LARVAE AND PUPAE OF ARMY-WORM IN NORTH CAROLINA IN 1914. Jour. Econ. Ent. 8: 299–302.
(15) TOWNSEND, C. H. T.
1895. CONTRIBUTIONS TO THE DIPTEROLOGY OF NORTH AMERICA. II. Trans. Amer. Ent. Soc. 22: 55–80.
(16) ———
1897. DIPTERA FROM THE LOWER RIO GRANDE OR TAMAULIPAN REGION OF TEXAS.—I. Jour. N. Y. Ent. Soc. 5: 171–178.
(17) ———
1911. ANNOUNCEMENT OF FURTHER RESULTS SECURED IN THE STUDY OF MUSCOID FLIES. Ann. Ent. Soc. Amer. 4: 127–152.
(18) VICKERY, R. A.
1915. NOTES ON THREE SPECIES OF HELIOPHILA WHICH INJURE CEREAL AND FORAGE CROPS AT BROWNSVILLE, TEXAS. Jour. Econ. Ent. 8: 389–392.
(19) WADLEY, F. M.
1921. LIFE HISTORY OF THE VARIEGATED CUTWORM. Jour. Econ. Ent. 14: 272–277.
(20) WAKELAND, C. C.
1920. VARIEGATED CUTWORM, PERIDROMA SAUCIA, HUBNER. Ann. Rpt. State Ent. Colo. (1919) 11: 17–19, illus.
(21) [WALKER, F.]
1849. LIST OF THE SPECIMENS OF DIPTEROUS INSECTS IN THE COLLECTION OF THE BRITISH MUSEUM. Part 4. London.
(22) ———
1856. INSECTA SAUNDERSIANA. v. 1, illus. London.

FURTHER STUDIES OF POTATO WILT CAUSED BY VERTICILLIUM ALBO-ATRUM[1]

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INTRODUCTION

In a previous paper on the transmission of some wilt diseases in seed potatoes (*3*)[2] it was pointed out that in western Oregon the wilt of potato plants caused by *Verticillium albo-atrum* Reinke and Berthold, is of more economic importance than that caused by *Fusarium oxysporum* Schlecht. In view of the limitations of our knowledge of the disease caused by *V. albo-atrum* it has been further studied, especially along the lines of methods of roguing to eliminate the organisms from seed potatoes and the transmission of the organisms through the soil, including length of rotation necessary for avoiding infection from the soil. The additional results secured in this study of Verticillium wilt conducted from 1916 to 1924 and now terminated are given herein. The methods employed in the work were outlined in the report mentioned above and are not repeated here.

TRANSMISSION OF THE ORGANISM IN SEED POTATOES

That *Verticillium albo-atrum* is transmitted in the seed potatoes from one crop to another has long been known, and methods have been recommended for the avoidance of this source of contamination. The methods that have been advocated and relied on chiefly in the past for this purpose are the discarding for seed of tubers showing distinct discoloration in the vascular region and the roguing from the seed plot of the individual plants showing wilt. That these methods are inadequate for successfully controlling the disease and that more care in the roguing of seed plots must be used are clearly shown by the results obtained in this investigation.

RELATION BETWEEN TUBER SYMPTOMS AND OCCURRENCE OF VERTICILLIUM

The inadvisability of depending on the presence or absence of discoloration as a guide to the presence or absence of wilt-producing fungi in potato tubers, which has already been shown for both *Verticilium albo-atrum* and *Fusarium oxysporum* (*3*), is further plainly indicated in a very striking way for *V. albo-atrum* by the data in Figure 1, presented here to bring briefly but clearly to mind the present status of information in regard to the relation of discoloration to the occurrence of *V. albo-atrum* in potato tubers. The two lots of tubers from which these results were obtained were grown from the two longitudinal halves of 272 tested wilt-free tubers, one lot

[1] Received for publication Mar. 30, 1925; issued February, 1926. Published with the permission of the director of the Oregon Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 470.

on soil infested by scattering Verticillium-diseased tops the previous fall and the other lot on clean well-rotated soil near by as a control. The discolored tubers grown by the wilt-infested plants on the contaminated soil gave a high percentage of infection with *V. albo-atrum*, whereas the discolored tubers grown by the wilt-free plants on clean soil showed no infection from this fungus. To make specific comparisons, 45.3 per cent of the tubers grown on contaminated soil were "browned" in the vascular region at the stem end, and of these 55.3 per cent gave *V. albo-atrum* and 7.2 per cent gave no organism in culture; 17.8 per cent of the tubers from the clean soil were "browned," but of these none gave *V. albo-atrum* and 48.3 per cent gave no organism in culture. A summary of all the discolored tubers from the two plots, listing the "end rot," "blackened," "browned," and "yellowed" tubers together as being discolored, shows that approximately 85 per cent of the tubers from the wilt-infested plot

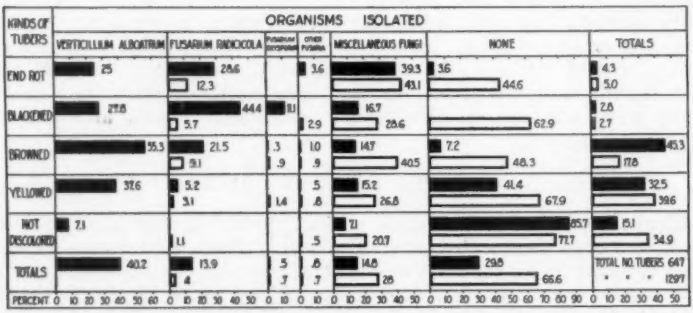
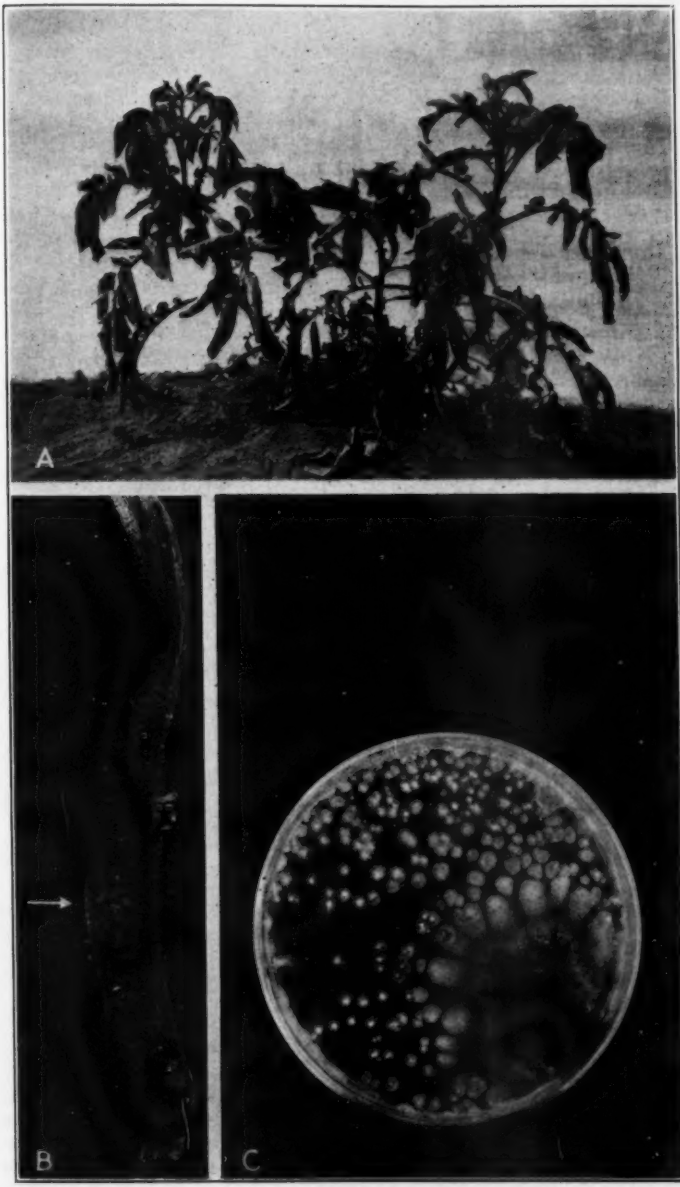


FIG. 1.—The relation between discoloration and the occurrence of various fungi in two lots of potato tubers. The tubers from which these results were obtained were grown from the two longitudinal halves of 272 tested wilt-free tubers, one lot, shown in solid black bars, on soil infested by scattering Verticillium-diseased tops the previous fall and the other lot, shown in outlined bars, on clean, well-rotated soil near by as a control

were discolored, of which 46 per cent were invaded by *V. albo-atrum*, and that approximately 65 per cent from the wilt-free plot were discolored, of which none were invaded by this fungus. The only reasonable conclusions from these data are that the presence or absence of *V. albo-atrum* in potato tubers is determined by the plants that produced them rather than by the presence or absence of tuber stem-end discoloration, and that tuber discoloration can not be relied on to any considerable extent for the separation of diseased from healthy tubers.

RESULTS OF PLANTING DISEASED SEED IN CLEAN SOIL

That *Verticillium albo-atrum* is readily transmitted from one season to the next in infected seed potatoes has already been demonstrated (*3*). For instance, one season's test showed 29.7 per cent infection of *V. albo-atrum* in tubers grown from seed potatoes infected by the same fungus when planted (pl. 1, A). One method that has frequently been recommended for lessening the danger of transmitting the organism in this way is the discarding of the stem end and planting only the eye end of the tubers for seed purposes.



A.—American Wonder potato plant wilting from effects of infection by *Verticillium albo-atrum* as a result of planting a diseased seed potato in clean soil. The fungus was isolated from the seed potato before planting and from one of the five tubers harvested. It also fruited on the base of the dead stalks during late summer. Photographed August 4, 1917

B.—Old potato stalk from a wilted plant after overwintering out of doors on the surface of the ground. This stalk was buried in a few inches of soil for about 10 days before it was brought to the laboratory on May 7, at which time it showed an abundant production of conidiophores and conidia of *Verticillium albo-atrum*. Much of the grayish surface growth especially abundant in the region near the arrow was caused by this fungus. Photographed May 7, 1917

C.—Petri-plate culture made from potato stalk overwintered at a depth of 3 inches in the soil. This culture was made May 11 with potato glucose agar to which was added at the time of pouring three drops of 10 per cent lactic acid. It contains approximately 200 colonies of *Verticillium albo-atrum*, and only a few colonies of other organisms

Some tests conducted by Pethybridge (*5*) have indicated that this wilt fungus is not localized at or near the stem end of affected tubers. In a test reported by the writer (*3*) where Verticillium-infested seed was used the stem-piece plants produced tubers bearing 22.7 per cent infection by *V. albo-atrum* and the eyepiece plants from the same tubers gave a crop with 24.6 per cent infection by the same fungus.

The fact that the method of discarding stem ends for wilt control still continues to be recommended led to the making of another test in 1923. In this case the seed used came from plants wilted by *Verticillium albo-atrum*, but the tubers themselves were not tested for the presence of this fungus before planting as was done in the former tests reported. The tubers, 100 in number, varying in size from 3 to 8 ounces, were cut crosswise into stem and eye halves of approximately equal size and were planted in pairs in rows side by side, the stem pieces in one row and the eyepieces in another row, on well-rotated land. Only a low percentage of wilt developed in the plants, because it was a little late in the season when these were planted and the weather was rather dry for a considerable period. However, the tubers produced by the plants gave, when harvested and cultured, 10.8 per cent Verticillium infection from the stem-piece plants and only 0.5 per cent from the eyepiece plants. From these figures it appears that some benefit might result from the use of eyepieces only for seed purposes. However, this practice would have a limited application only, being advisable in special cases, as for instance, in seed plots where no special precautions have previously been taken to eliminate wilt from the seed stock. In any event, though where the grower is willing to go to the slight extra trouble there would be no objection to the use of only eye-end pieces in the seed plot. One should, however, guard against placing too much reliance on this as an effective method for wilt control. In seed stocks that have been grown with careful roguing and other effective practices in use, it would probably be unnecessary and inadvisable to discard stem ends and plant only eye-end pieces for Verticillium wilt control.

SPREAD OF THE DISEASE FROM PLANT TO PLANT IN THE ROW

As reported in 1921 (*3*) in the work on the transmission of *Verticillium albo-atrum* in seed potatoes, some of the plants grown on clean soil from tubers from which other organisms than *V. albo-atrum* or no organisms were isolated before planting also gave a rather large percentage of Verticillium infection in the yields. For instance, in the 1917 tests plants grown on clean soil from Verticillium-infected seed gave 29.7 per cent of Verticillium infection in the yields; the seed potatoes from which only "miscellaneous fungi" were isolated gave 13.3 per cent, and those from which no organisms were isolated gave 18.9 per cent. The occurrence of Verticillium in the tubers from these other plants can be explained from knowledge we now have of the spread of the organism from one plant to another in the row during the growing season. At the time these plants were grown it was not known or surmised that Verticillium ever spread in this way, so no precautions had been taken to avoid it. The seed tubers from which these results were secured were planted about 15 inches apart in the row according to the plant by which grown and the size of the

tubers, and not according to the organism isolated from them before planting. Thus it frequently happened that a Verticillium-infected seed potato was planted next to a noninfected tuber and if the fungus had any inclination to spread from one plant to another in the row it had an excellent opportunity to exercise this propensity.

When the data on the yields from these plants were tabulated on the basis of distance of the plants in the row from known Verticillium-infected seed (Table I) it was found that in general infection from Verticillium in the yields ran highest in the plants located next to a plant grown from a Verticillium-infected seed potato and dropped off to a very low percentage in the tubers from the fifth plants away and beyond, the percentages for plants 1 to 5 and beyond being 35.2, 29.4, 31.5, 14.5, and 3, respectively. These figures seem to indicate that Verticillium had spread commonly to the third plant away but not much beyond. It should be added here that the season in which these potatoes were grown was a favorable one for the development of Verticillium wilt and also that no tests on distance of spread conducted since that time in less favorable years have given results that exceeded or even quite equaled those just reported.

Since this spread of the disease to the neighboring plants was observed most commonly in those nearest by, and to only a slight extent in those farther away, it was thought likely that the transfer of the fungus from one plant to another took place between the root systems of the plants in contact with each other. Another possible means of spread is through the scattering of conidia after the fungus fruits on the wilt-affected plants. Since the conidia are usually not produced in abundance on the wilted plants until the fall rains begin, and this is commonly after the crop is mature or nearly mature, it does not seem probable that spread is effected in this way. Also if spread were accomplished by means of the conidia the distribution of the disease seemingly would be more irregular and not limited so closely to the adjoining plants in the same row. The exact manner in which this spread between plants is accomplished has not been determined, though all the evidence available and presented here leads to the conclusion that it is brought about through the intermingling of the root systems.

In 1919 a field test was conducted especially to secure additional evidence on the extent of spread of Verticillium from plant to plant during the growing season. Well-selected seed potatoes known to be quite wilt-free were planted May 1 on well-rotated land in rows 39 inches apart and at regular intervals of 15 inches in the row. In 15 of the 17 rows, one out of every six seed pieces was inoculated by placing on top of it a culture of *V. albo-atrum* growing on sweet-clover stems. These pieces were then covered with soil in the furrow and so placed as to give when the plants were produced one original wilt-infected plant and five healthy plants next in the row. Altogether 103 seed pieces were inoculated and a total of 618 hills were planted in the whole plot besides the two rows left as controls in which no inoculations were made and the border hills at either end of the plot. Figure 2 shows the plan of a part of the plot and indicates also the condition of the plants with reference to the incidence of wilt as noted in this part of the plot on August 12.

TABLE I.—*Evidence of the spread of Verticillium albo-atrum from plant to plant in the field*

[The plants are grouped according to whether or not *Verticillium albo-atrum* was isolated from the seed potatoes from which they were grown. Those plants from whose seed tubers *V. albo-atrum* was not isolated are grouped according to the distance at which they were grown from a plant from whose seed tuber *V. albo-atrum* was isolated before planting. Nine varieties of potatoes were grown in the tests. Season of 1917]

	Plants grown from seed tubers from which V. albo-atrum was isolated before planting, showing—		Plants grown from seed tubers from which V. albo-atrum was not isolated before planting, showing—										Total	
			[a] 1		[a] 2		[a] 3		[a] 4		[a] 5 and beyond			
	Infected yields	No infection in yields	Infected yields	No infection in yields	Infected yields	No infection in yields	Infected yields	No infection in yields	Infected yields	No infection in yields	Infected yields	No infection in yields	Infected yields	No infection in yields
Number of hills	105	46	70	28	19	16	8	8	6	3	28	291	130	347
Per cent of infected hills	69.5		71.4		54.3		50.0		66.7		8.8		27.3	
Number of tubers in yields	[b] +325 [b] −430	−339	+251 −298	−165	+80 −81	−111	+39 −35	−50	[b] +12 [b] −39	−32	+65 −157	−1,949	+447 −610	−2,307
Per cent of infected tubers in infected hills	43.0		45.8		49.7		52.7		23.5		29.3		42.3	
Per cent of infected tubers in all hills	29.7		35.2		29.4		31.5		14.5		3.0		13.3	

[a] Number of plants distant from a plant grown from a tuber from which *V. albo-atrum* was isolated before planting.
[b] The plus (+) sign indicates the tubers in the yields that were infected with *Verticillium albo-atrum* and the minus (−) sign those that were not infected as shown by cultures made therefrom.

TABLE II.—*Results secured from plot for determining spread of Verticillium from plant to plant in the row; season of 1919; variety, Up-to-Date (100 plants inoculated)*

	Plants inoculated	Number of plant distant from inoculated plant				
		1	2	3	4	5
Number of plants in test	100	102	102	101	103	101
Per cent wilt, as noted in plants July 11	86.6	0.0	1.0	0.0	0.0	0.0
Per cent wilt, as noted in plants Aug. 12	98.0	28.1	5.8	1.9	2.9	0.0
Number of tubers produced per plant	4.8	6.0	6.6	6.6	6.9	6.9
Weight in ounces of tubers per plant	5.5	13.9	19.0	19.4	19.5	20.3

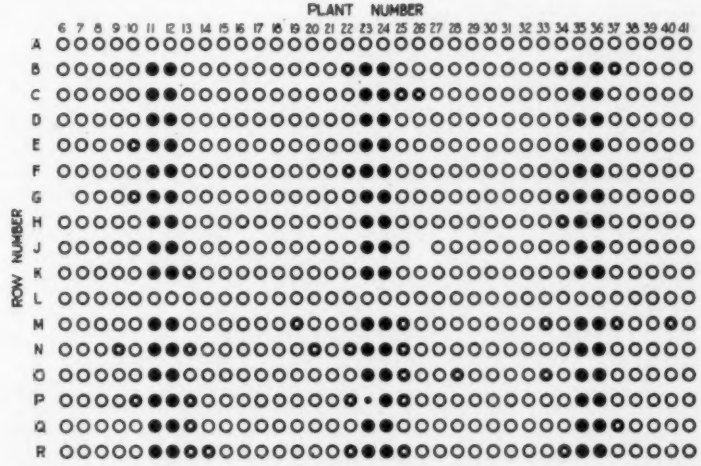


FIG. 2.—Plan of a portion of the 1919 plot for determining the spread of *Verticillium albo-atrum* from plant to plant in the row. The condition of the plants as noted on August 12 is indicated by symbols as follows: Plain circle, as A 6, indicates healthy uninoculated plant; solid circle, as B 11, inoculated wilted plant; heavy circle with small white center, as E 10, uninoculated wilted plant; and small black circle, as P 23, inoculated seed piece which failed to produce a plant

In Table II is given a summary of the occurrence of wilt in the different plants on July 11 and August 12 and the number and total weight of tubers produced per hill at harvest time in October. No complete notes were made on the growth of the plants after August 12, since it was difficult to distinguish accurately between wilt symptoms and natural maturity, and, furthermore, it was anticipated that cultures of the tubers for Verticillium to be made after harvest would give valuable supplementary evidence on the extent to which the fungus had spread among the plants. Unfortunately the tubers were never tested for Verticillium infection after harvest, for the entire crop was lost while in storage owing to an exceptionally heavy freeze in December. In this case, then, judgment on the extent of spread must be made on plant appearance and yield records. On July 11, 86.6 per cent of the plants from the inoculated tubers showed

distinct evidence of wilt infection (pl. 2, B, left), one plant, or 1 per cent of plants No. 2 in the row from the inoculated tubers, showed wilt; none of the other plants showed any. On August 12, 98 per cent of the plants from the inoculated tubers were either dead from wilt or showed distinct wilt; 28.1 per cent of plants No. 1 (pl. 2, C), 5.8 per cent of plants No. 2, 1.9 per cent of plants No. 3, 2.9 per cent of plants No. 4, and none of plants No. 5 showed wilt. Thus the disease had apparently spread to a considerable extent to the second plant away in the row. If the tubers from all of these plants could have been tested they no doubt would have showed a wider spread of the fungus than was revealed by the appearance of the plants alone. The wilt had a depressing effect on the yields, being manifested most, of course, in the inoculated lot, clearly evident in plants No. 1 and possibly exerting some influence in plants No. 2, the extent of depression being much more pronounced in the weight than in the number of tubers produced per hill.

ROGUING METHODS FOR ELIMINATION OF FIELD INFECTION

When it was realized that Verticillium does spread commonly from plant to plant in the field, it was naturally wondered to what extent this could be avoided by the roguing out of affected plants as they were observed during the growing season. To get information on this point a field test was conducted in 1920, similar to that of 1919 just reported, except that instead of one plot three plots were planted in close proximity. In one plot notes were kept on the growth of the plants but no roguing of the wilted plants was made. In the second plot all wilted plants were rogued out promptly as noted. In the third plot the wilted plants, and also the next healthy plant on either side of the wilted one, were rogued at the same time. For convenience this is termed the three-plant method of roguing. These plots were gone over seven times from July 9 to August 28. After August 28 wilt could not be clearly enough distinguished from maturity to justify additional roguing. The season, being unusually dry from planting time on, was so abnormal for wilt development that only about 43 per cent of the plants from the inoculated tubers showed distinct symptoms of wilt. In view of this lack of normal wilt development in the inoculated lot the evidence secured on the value of roguing could not be accepted as indicative of average conditions, and so it was deemed advisable to duplicate the tests the following year. Figure 3 shows the plan of a part of the 1921 plots to determine the value of roguing for Verticillium control, and indicates the plants which, as judged by appearances, had been affected with wilt by August 22. It shows also the total tubers produced per hill and the number of tubers from which *V. albo-atrum* was isolated in cultures made after harvest. These plots were gone over five times from July 11 to August 22, after which time no more roguing was done. The crop was harvested October 4, and the individual tubers were tested for infection with *V. albo-atrum* by the sweet-clover stem-culture method previously described.

In the plot where no roguing was done the plants which showed wilt symptoms during the growing season gave 40 per cent infection of *Verticillium albo-atrum* in the tubers (Table III and fig. 4), those

Spread of *Verticillium albo-atrum* from one plant to another in the row. Variety, Up-to-Date

A.—Plant at left grown from diseased seed tuber, at right from healthy seed tuber. Though the plant at the right showed no indication of infection from wilt during the growing season, aside from possibly slight premature ripening, yet 10 of the 22 tubers produced, or 45 per cent, gave *Verticillium albo-atrum* in culture when tested after harvest. Infection of this plant, without question, came from the adjoining diseased plant. Photographed August 22, 1917

B.—Plant at left showing wilt by drooping and loss of color in some leaves and by undersize resulting from inoculation with *Verticillium albo-atrum* at planting time; plant at right healthy in appearance, not inoculated. Photographed July 12, 1919

C.—Same two plants as in B later in the season. Inoculated plant at left nearly dead from wilt, uninoculated plant at right showing unmistakable symptoms of infection with wilt in the drooping and dying of some of the lower leaves. Infection of this plant presumably took place through the root system from contact with the neighboring diseased plant. Photographed August 13, 1919

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plants located next to a wilted plant but not themselves showing wilt gave 19 per cent infection in the yields, those situated two plants away gave 4.5 per cent, and those three plants away gave 0.7 per cent. These records are in general harmony with those of former

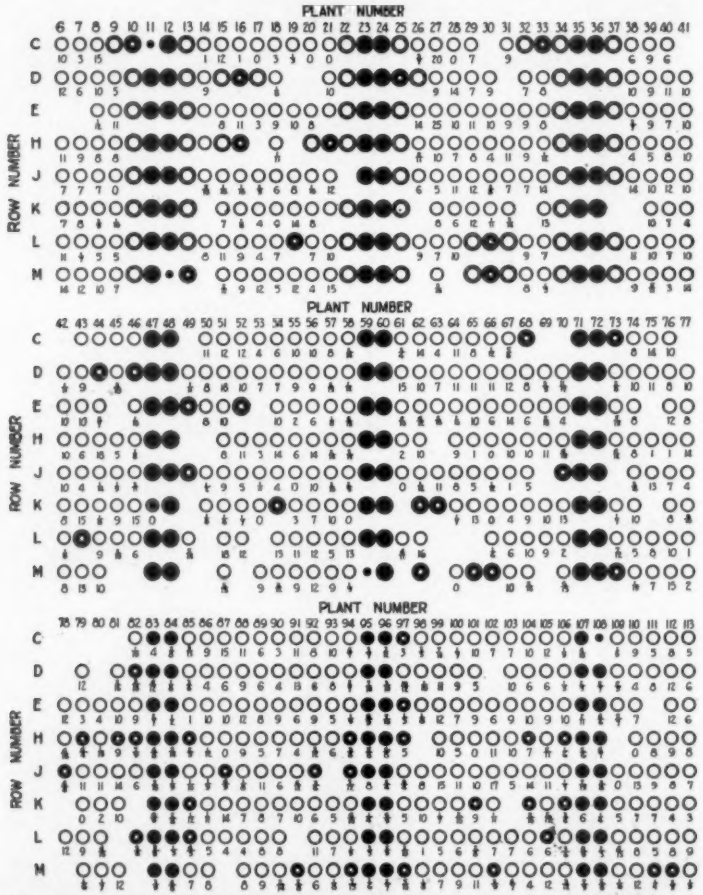


FIG. 3.—Plan of a portion of the 1921 plots for determining the spread of Verticillium from plant to plant in the row and the influence of the one and three plant methods of roguing thereon. The appearance of the plants as noted during the season up to August 22 and the condition of the tubers harvested are indicated by symbols as follows: Plain circle, as C 82, indicates healthy uninoculated plant; solid circle, as C 84, inoculated wilted plant; heavy circle with small white center, as D 82, uninoculated wilted plant; small black circle, as C 108, inoculated seed piece which failed to produce a plant; additional outer circle, as C 9, C 12, plant rogued during growing season; whole number below circle, total tubers produced of which none yielded Verticillium when cultured; and fractional number below circle, number of tubers yielding cultures of *Verticillium albo-atrum* out of the total tubers produced. A summary of these results is given in Table III and Figure 4

years, and indicate extensive contamination of tubers in hills healthy in appearance but adjoining visibly wilt-affected plants and noticeable spread of the organism to the tubers of the second plant in the row from a wilted plant.

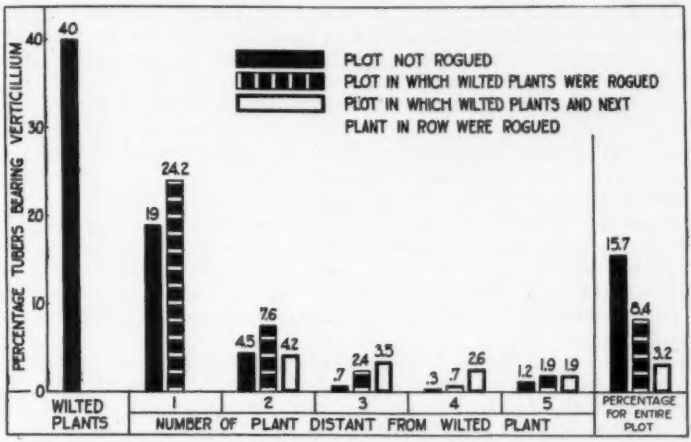


FIG. 4.—Graph showing a summary of the results secured from the 1921 plots for determining the spread of *Verticillium albo-atrum* from plant to plant in the row and the influence of the one and three plant methods of roguing thereon

TABLE III.—*Results obtained from roguing plots for control of spread of Verticillium albo-atrum from plant to plant in the field; variety, Up-to-Date; season of 1921; Corvallis, Oreg.*

	Plants showing wilt in the field				Plants showing no wilt in the field and located at different distances in the row from plants visibly affected with wilt							
					Plant No. 1				Plant No. 2			
	Number of hills	Number of tubers produced	Tubers infected with V. albo-atrum	Percentage of infected tubers	Number of hills	Number of tubers produced	Tubers infected with V. albo-atrum	Percentage of infected tubers	Number of hills	Number of tubers produced	Tubers infected with V. albo-atrum	Percentage of infected tubers
Not rogued	74	567	227	40.0	54	474	90	19.0	46	396	18	4.5
Wilted plants rogued	(63)*				38	389	94	24.2	45	432	33	7.6
Wilted plants and next healthy plant on either side in same row rogued	(56)				(54)				42	383	16	4.2

	Plants showing no wilt in the field and located at different distances in the row from plants visibly affected with wilt											
	Plant No. 3				Plant No. 4				Plant No. 5			
	Number of hills	Number of tubers produced	Tubers infected with V. albo-atrum	Percentage of infected tubers	Number of hills	Number of tubers produced	Tubers infected with V. albo-atrum	Percentage of infected tubers	Number of hills	Number of tubers produced	Tubers infected with V. albo-atrum	Percentage of infected tubers
Not rogued	35	280	2	0.7	32	282	1	0.35	22	166	2	1.2
Wilted plants rogued	38	369	9	2.4	32	290	2	.7	27	214	4	1.9
Wilted plants and next healthy plant on either side in same row rogued	40	370	13	3.5	31	266	7	2.6	29	260	5	1.9

* Number in parentheses shows the number of plants rogued.

In the second plot, where all plants showing wilt were rogued as soon as it was noted, the adjoining plants in the row gave 24.2 per cent infection in the yields, the second plants away gave 7.6 per cent, and the third plants 2.4 per cent.

In the third plot, where the wilted plants and the next healthy plant on either side in the same row were rogued at the same time, that is, where the three-plant method of roguing was used, the second plant away from the plant which showed wilt gave 4.2 per cent and the third plant gave 3.5 per cent infection in the yields. The variations in the amount of wilt infection present in the three plots in the tubers of plants Nos. 3, 4, and 5 grouped together—the figures being 0.7, 1.7, and 2.7 per cent for the nonrogued, one-plant rogued, and three-plant rogued plots, respectively—are taken as being due chiefly to experimental error and are not considered as having any special significance.

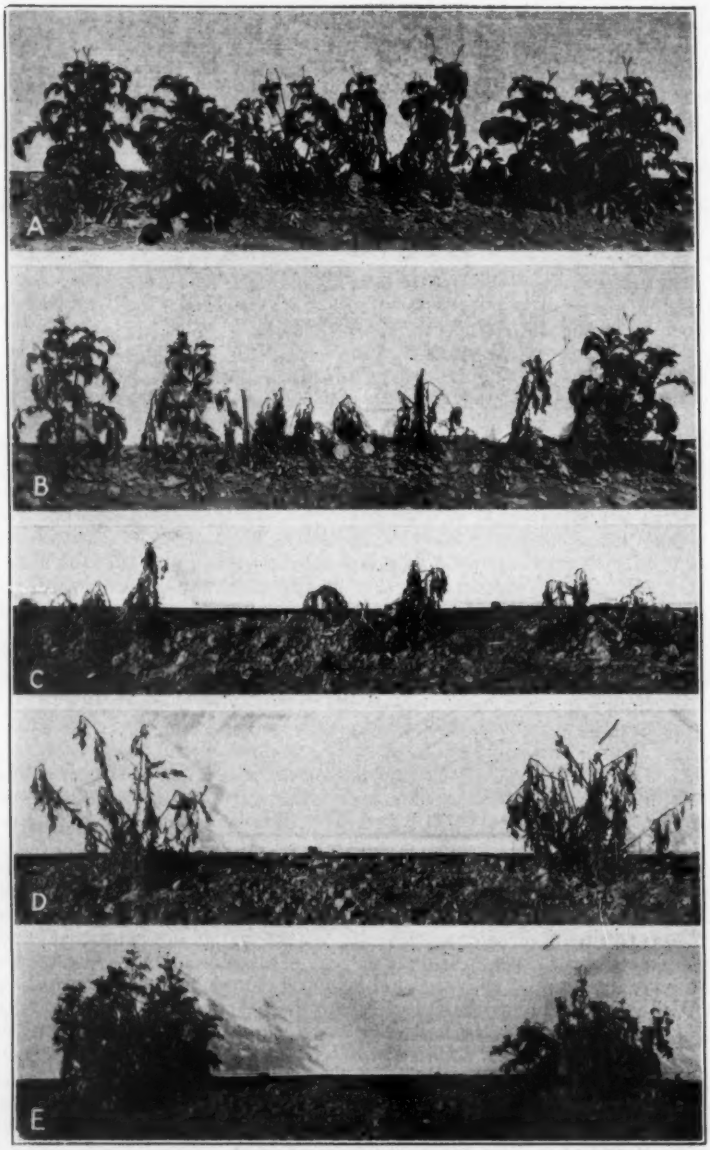
It should be mentioned that some of the plants grown in the plots discussed above may have become infected directly from the inoculum itself which was placed in contact with the neighboring seed piece rather than from the spread of the fungus from the affected plants. This would be particularly true of plants "No. 1," that is, the plants immediately adjoining the inoculated plants. Although there is no way of determining whether direct infection of plants "No. 1" did occur, it is not believed that it took place to any considerable extent, because the presence of the root system of the inoculated plant would prevent any appreciable growth of the roots of plant No. 1 in the area where the inoculum was placed. In the case of plants "No. 2" direct infection would be inconceivable, for competition between the root systems would effectively prevent the roots from plants "No. 2" ever growing past the two other plants and to the distance of 30 inches necessary to come in direct contact with the inoculum. That infection does spread from one plant to another under natural conditions is well shown by the data in Table I, in which case no artificial inoculum had been introduced. Natural spread has also been observed many times in the field (pl. 2, A).

There is a practical interest in knowing, too, whether roguing of diseased plants actually checks the spread or increases the chances of spread of the fungus to the adjoining plants. It is perhaps worth noting here that, in the plot where all noticeably wilt-affected plants were rogued, plants "No. 1" gave a higher percentage of infected tubers than did plants "No. 1" in the nonrogued plot, 24.2 and 19 per cent, respectively. It seems reasonable to suppose that where diseased plants are rogued the roots of adjoining plants would grow more freely into the area formerly occupied by the diseased plant, and since in roguing diseased plants many Verticillium-invaded roots would be broken off and left in the ground the chances of spread might be increased rather than decreased. This influence, if there is one, would be expressed more plainly with the one-plant method than with the three-plant method of roguing. There are not sufficient data at hand to furnish an adequate answer to this question. The most that can be said is that the figures from these plots do not seem to betoken any special advantage in roguing wilted or wilted and adjoining plants as compared to the elimination of comparable plants by staking and digging separately at harvest time.

The figures secured indicate a very decided advantage of the three-plant method of roguing over the ordinary roguing of only such plants as can be detected on appearance to be wilt-infected. This is evident from the high infection of the tubers in the plants adjoining visibly wilted plants and is also well shown by the percentages of Verticillium in the tubers remaining in the plots following the two methods of roguing. For instance, in the second plot, where only visibly wilt-affected plants were rogued, 24.2 per cent of the tubers from the next adjoining hills were infected and a total of 8.4 per cent infection was secured in the tubers harvested from the entire plot, whereas in the third plot, where the wilted and adjoining plants were rogued, only 3.2 per cent infection remained in the tubers harvested from the entire plot. In this latter case, following the three-plant method of roguing, this represents a reduction of from slightly more than 22 per cent visible wilt infection in the plants to 3.2 per cent infection in the tubers in one season's time.

Plate 3, A and B, shows six plants from the nonrogued plot photographed August 1, when only the inoculated hills had wilt, and again on August 31, at which time the two adjoining plants on one side and plant No. 1 on the other of the inoculated hills were plainly wilt diseased. It is worthy of note that the one plant in this illustration which did not show wilt in the field gave one infected tuber out of the 12 produced.

From the above data it is clearly apparent that not all plants that produce infected yields show visible symptoms of wilt infection in the field. A great many plants have been grown at different times that exhibited no detectable wilt in the field but which produced tubers containing a high percentage of Verticillium infection. This is to be explained, no doubt, on the basis that the effects of the disease on the plant itself were so slight as to escape detection or, more likely, that infection took place so late in the season as to cause little or no change in the appearance of the plant, or to make it impossible to distinguish clearly between infection and normal maturity of the vines. Verticillium infection of young stolons has been encountered (pl. 7, E) as early as July 9, just two months after planting, but it is not known how late infection may gain entrance into plants and grow on into the tubers. It is believed, however, that this may take place quite late in the season, possibly nearly up to harvest time. The plant at the right in Plate 2, A, grown from a Verticillium-free seed potato but next to a wilted plant grown from a naturally infected seed tuber, showed no distinct wilt symptoms aside from possible slight premature ripening, and yet 45 per cent of the tubers produced were infected when harvested and cultured. This plant was grown before it was realized that the fungus spreads from one plant to another, and it was not known to have been affected with wilt in any way until the tubers were cultured after harvest. The notes taken on the plants during the season then recalled the fact that it had apparently matured a little in advance of some, but not many, of the plants in the same field. To make a common practice of the three-plant method of roguing would eliminate the great majority of this type of infection and in a short time would result in the reduction of damage from Verticillium to a negligible quantity.



For explanatory legend see p. 45.

In many fields in western Oregon there is frequently considerable variation in the final maturing of the different plants in the same field that is not associated in any way with the occurrence of the wilt disease. This, however, ordinarily need not cause confusion in roguing, since roguing should be applied only to the visibly wilt-affected and the adjoining plants in the same row. Where Verticillium is present in the field it commonly makes itself clearly evident sufficiently far in advance of maturity to permit of close but not excessive roguing.

In spite of the fact that much evidence has been secured on the spread of Verticillium from plant to plant within the same row, the evidence collected during the same period on the spread of the organism from one row to another has been strikingly negative. In 1917, when the most extensive spread of Verticillium within the row was secured, a row of Verticillium-free seed potatoes produced only five Verticillium-infected tubers, or 0.8 per cent infection, out of a total of 645 tubers, whereas the adjoining row composed of Verticillium-wilted plants gave 49 per cent Verticillium infection in the yields. These rows were grown, as usual, about 39 inches apart. In the spread-of-Verticillium plots in 1919 there was no evidence whatever that this fungus was able to spread to the adjoining row (fig. 2).

Assuming that the spread of the disease from plant to plant is accomplished through contact of the root systems, the two factors that suggest themselves as being unfavorable to the spread of the organism from one row to another are the distance of the rows apart and the prevention of the free and extensive intermingling of the roots by cultivation. Personally, the writer doubts whether the distance of the rows apart would in itself prevent the spread of the disease to the adjoining row under favorable circumstances. It would seem that the checking of the mingling of the root systems by cultivation would be more effective. In this connection it might be well to state that though no specific experiments have been conducted to test the opinion, it is believed that the planting of the hills in checkrows

EXPLANATORY LEGEND FOR PLATE 3

A and B.—Spread of *Verticillium albo-atrum* from one plant to another in the row as illustrated by a group of plants numbered from left to right H 81 to H 86 in Figure 3, season of 1921. Variety, Up-to-Date

A.—The two central plants were inoculated and show distinct wilt. The others were not inoculated and none are showing wilt. Photographed August 1

B.—Same plants as in A photographed later in the season, August 31. The two central inoculated plants are dead from wilt, and three of the others though not inoculated are showing wilt. The two plants adjoining the original wilted plants are themselves badly diseased. Of the second plants distant in the row only the one at the left is showing disease in the wilting and dying of the lower leaves, the other at the right does not exhibit wilt symptoms here, but it is worth noting that one tuber out of the 12 produced by it gave *Verticillium albo-atrum* when cultured after harvest. From left to right the tubers from these six plants gave when cultured after harvest none carrying *V. albo-atrum* out of a total of 9 produced, 7 out of a total of 9, 4 out of 8, 3 out of 14, 2 out of 5, and 1 out of 12, respectively. Thus spread of the disease to the second plant away is indicated

C, D, and E.—Distance of inoculum of *Verticillium albo-atrum* from the seed piece. Photographed September 28, 1923. Variety, Up-to-Date

C.—Three representative plants which died rather early in the season from wilt infection resulting from inoculum placed in the soil at a distance of 15 inches from the seed pieces. The tubers from these plants from left to right gave when cultured after harvest 5 infected with *Verticillium albo-atrum* out of 7 produced, 3 out of 8, and 2 out of 6, respectively

D.—Two wilted plants from the plot inoculated by placing the inoculum 30 inches from the seed pieces. These plants grew considerably larger and died later than those in the 15-inch plot. The tubers from the plant at the left showed no infection by *Verticillium albo-atrum* when cultured after harvest and those from the one at the right gave two infected tubers out of eight produced

E.—Two plants, one healthy in appearance and the other showing distinct wilt, from the plot in which the inoculum was placed in the soil 45 inches from the seed pieces. As would be expected, these plants remained healthy much longer than those where the inoculum was placed closer to the seed pieces. The plant at the left gave 3 tubers infected by *Verticillium albo-atrum* out of 14 produced and the one at the right gave 5 out of 9

to permit of cultivation both ways, a planting method occasionally used by some growers, would eliminate the necessity of roguing more than just the plants visibly affected with wilt. In a later paragraph will be found more data on the maximum distance from plants at which the inoculum may be placed and still give infection with Verticillium. The evidence there presented leads to the belief that infection may be secured when the fungus is at a distance of 45 inches from the seed piece if the free spread of the roots is not hindered by cultivation.

In connection with the testing of tubers from hills to determine the extent of spread of the organism from plant to plant, an effort was made to secure an idea of the value of cultures made from the individual tubers for the determination of the percentage of wilt infection as compared to a planting test of the tubers in the field and noting for the development of wilt. For this comparison the tubers from six rows of the 1921 Verticillium-spread plots were cultured in the laboratory, and from these were secured the data discussed in Table III. The tubers from two rows of the same plots were planted without culturing in the field the following season. Watch was kept for the development of wilt in the resulting plants, and late in September notes were made of the plants on which Verticillium was found fruiting. The season was too dry for the best growth of the plants, and it was found impossible to make a close determination of the presence of wilt symptoms. The growth of the fungus on the dead plants could be accepted with some assurance for a positive diagnosis but was a less reliable criterion for negative determinations, for at the time these notes were made the fungus had not yet fruited on all wilt-affected plants because of the lack of abundant soil moisture. The results from the field test indicate spread of the fungus among the plants the previous year, much as was revealed by the tuber cultures made from the comparable plants, but to a much less degree. The tuber culture test for determining the percentage of tuber infection proved far superior to the field test, chiefly for the following reasons. It is more cheaply, easily, and quickly made. It is more reliable, for fewer uncontrolled factors are encountered. In the field some plants are unavoidably lost through rotting, occurrence of other diseases, destruction of the seed piece by field mice or gophers, etc. Not all plants grown from diseased seed develop the disease. In a season unfavorable for wilt this may become a large factor. In mild cases of the disease or in case of late infections the symptoms are not distinct enough to permit accurate diagnosis. Unless the tubers are planted far apart the disease may spread from one plant to another and complicate the results.

TRANSMISSION OF THE ORGANISM IN THE SOIL

The utilization of crop rotations to avoid infection of *Verticillium albo-atrum* from the soil has been commonly appreciated and long used. However, there does not appear to have been many data collected on the extent to which the fungus may be harbored in the soil or how long the rotations must be made to effect control. Nevertheless, some data have been collected that are of aid in formulating dependable farm practices. Naturally, not all of the questions have

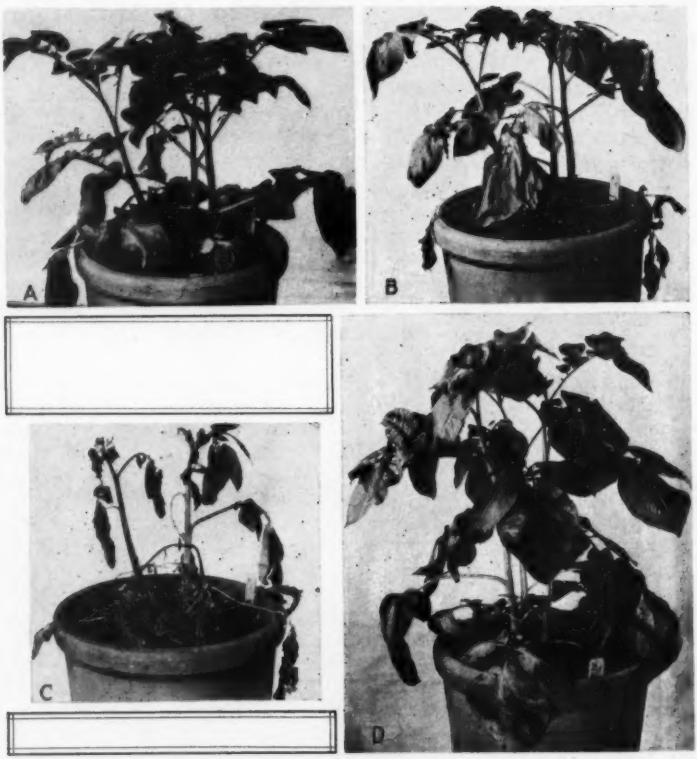
yet been answered, but enough information has been secured apparently to justify some conclusions at the present time. In this section will be presented some experimental data which lead to the conclusions that infection of potato plants takes place very readily from contaminated soil, that such infection gains entrance largely, if not entirely, through the root systems, and that a three-year rotation is sufficient to avoid infections from the soil. A longer rotation than this would be necessary only if potatoes were held over excessively in the soil as volunteers or out of consideration to other diseases to which the potato is subject.

INFECTION OF PLANTS FROM CONTAMINATED SOIL

Early in the investigation some tests were conducted to determine the ability of the organism in the soil to gain entrance to plants growing therein and cause wilt. On December 21, 1916, stalks collected from potato hills showing wilt at the previous harvest and bearing *Verticillium albo-atrum* in a fruiting condition were cut into small pieces and mixed with soil previously treated with a solution of formaldehyde and well aired out. This contaminated soil was then placed in an open pit out of doors subject to natural weather conditions.

On January 24 the soil was brought into the greenhouse and potted. Fifteen tested wilt-free tubers were cut longitudinally and planted, one half of each tuber in infested soil and the other half in clean uninfested soil as a control. Notes were made at various times during the growth of the plants, those made April 20 showing distinct wilt symptoms in 53 per cent of the plants in the contaminated soil and no wilt in the clean soil. On May 26, when the plants were dug, 93 per cent of those grown on the contaminated soil had Verticillium fruiting on some portion of the surface, generally on the main stalk at or a little above the soil line, whereas none of those on the clean soil showed this fungus. Of the 124 tubers produced in the contaminated soil, 10.5 per cent gave *V. albo-atrum* in culture when tested by the sweet-clover-stem method and none of the tubers from the control plants gave this fungus in culture. Seven varieties of potatoes, namely, American Wonder, Burbank, Irish Cobbler, Early Rose, Gold Coin, Rural, and Uncle Sam, were used in these tests. There was no apparent difference in the reaction of the varieties to the disease.

Concurrently with the above test was run another similar trial, except in this case the inoculum used was a 34-day-old culture of the fungus growing on old sterilized potato stalks. About a tablespoonful of this material cut into small pieces was placed on the seed piece before covering it with soil on January 24. The disease developed at about the same rate and extent as in the test reported above. On April 20, 60 per cent of the plants from the inoculated lot were showing distinct wilt symptoms. When dug on May 26, 80 per cent of these plants showed *Verticillium albo-atrum* fruiting on the surface of the stalks, and the tubers when cultured gave 7.1 per cent wilt infection. The plants grown from the other halves of the same seed tubers on clean soil as controls developed no wilt symptoms, showed no growth of the fungus on the stalks at harvest time, and gave no evidence of infection in the tubers when these were cultured.



Wilt of potato plant in the greenhouse as a result of inoculation by placing on the seed piece a 34-day-old pure culture of *Verticillium albo-atrum* on old sterilized potato stalks before covering with soil, January 24, 1917. Variety, Irish Cobbler

A.—Plant from inoculated seed piece showing first symptoms of wilt in the premature yellowing and dying of the lower, oldest leaves. Photographed March 27

B.—Same plant as in A, 17 days later with wilt symptoms clearly exhibited. Photographed April 13

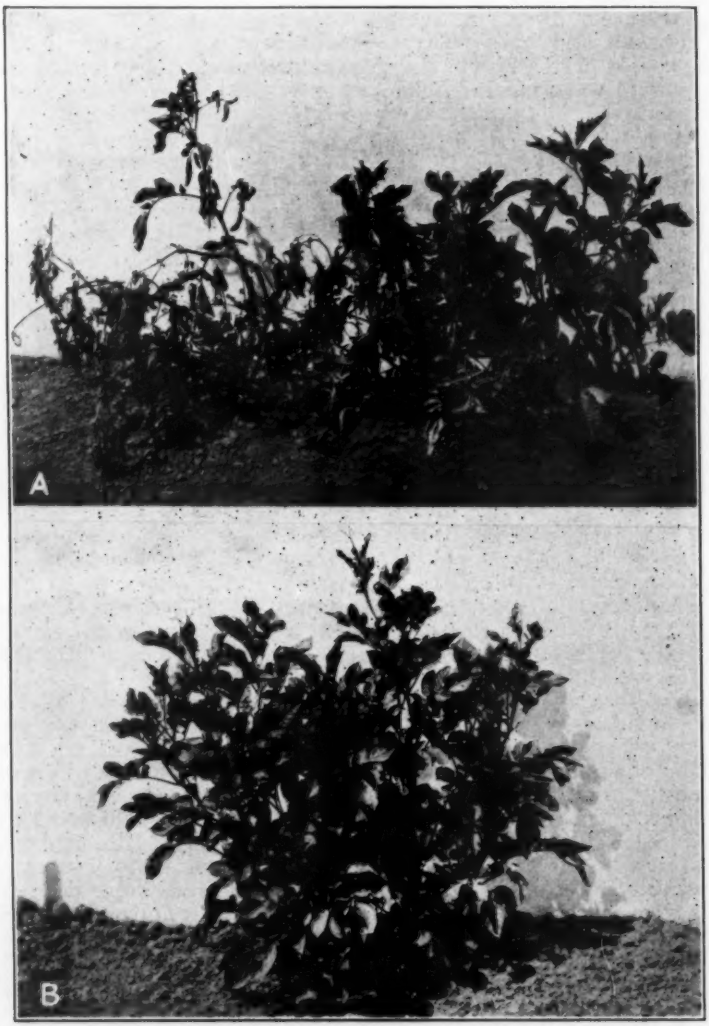
C.—Same plant as in B, 10 days later, plant nearly dead with wilt. When dug, *Verticillium albo-atrum* was found fruiting on one stalk of this plant. One of the seven tubers produced in the hill also gave this fungus when cultured. Photographed April 23

D.—Uninoculated plant grown on adjoining bench in same greenhouse from other half of same seed tuber used to produce plant A–B–C above. No wilt symptoms developed on this plant during growth, and none of the tubers produced gave this fungus in culture when tested. Plant same age as C above. Photographed April 23

In Plate 4 is illustrated one pair of Irish Cobbler plants from this test, showing the progress of the wilt in the inoculated plant and the absence of disease in the control plant grown from the other half of the same seed tuber. The inoculated plant (pl. 4, A) when photographed on March 27 was showing the first symptoms of wilt in the premature yellowing and dying of the terminal leaflets of the lower, oldest leaves. Three of the oldest leaves had died and fallen. The terminal leaflets of the three next oldest leaves were yellow and those of the next four oldest leaves, including the one extending out directly over the pot label, were yellowish green. The rest of the plant was green. These first symptoms have frequently been noticed on plants developing wilt. From this time on the leaves gradually wilted and died until April 23, when the plant was practically dead. At harvest time *Verticellium albo-atrum* was found fruiting on one stalk of this plant. One of the seven tubers produced in the hill also gave this fungus when cultured.

Next, some inoculations were made in the field to test for infection under more natural conditions. Seventy-eight tested wilt-free tubers of the same seven varieties as used in the greenhouse tests just mentioned and one additional variety, Up-to-Date, or "Pacific White Rose," as called by some, were divided longitudinally into halves and planted on May 1, 1917, in two plots. The plots were located a short distance apart on soil which had been well rotated and which had not grown potatoes for at least five years. In one plot inoculations were made by placing on top of each seed piece a culture of *Verticillium albo-atrum* growing on sweet-clover stems and then covering them with soil in the furrow at the usual depth. The other plot was left uninoculated as a control. Infection took place very readily from the soil (pl. 5, A, B), for 99 per cent of the plants on the inoculated plot were affected by wilt before the end of the season and 49 per cent of the tubers produced gave *V. albo-atrum* when cultured after harvest. None of the plants on the control plot were found wilted and none of the tubers tested gave *V. albo-atrum* in culture.

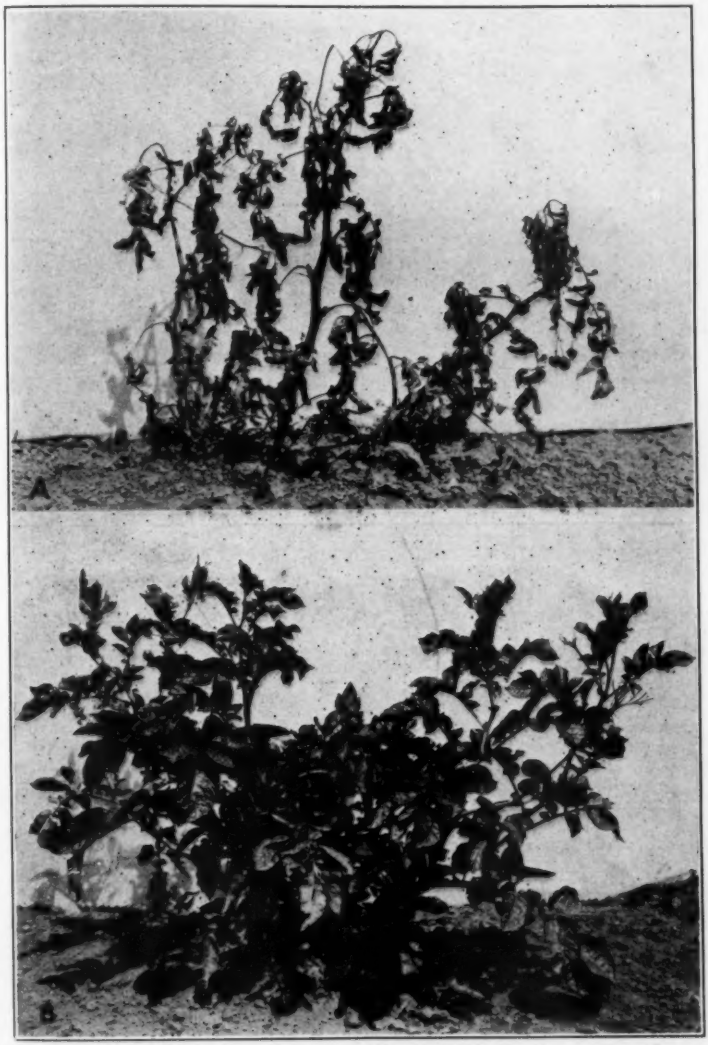
One other field test to demonstrate infection from the soil was conducted in which old diseased potato tops were used as the source of the organism in the soil. Search failed to locate a field that had produced a diseased crop the year before which could be used for the test without introducing too many undesirable factors, so as a substitute, in the fall of 1916, potato vines from Verticillium-diseased hills were collected from various sources and scattered over a plot of ground suitable for the test. The ground selected had not grown potatoes recently and had been well rotated. The old diseased potato tops were scattered at the rate of about one stalk for every 4 square feet. In the early winter this ground was double disked and in the spring was plowed and prepared for planting. The seed potatoes selected were produced by plants which showed no wilt during growth, and none of these tubers had given growth of Verticillium when cultured. The seed tubers, 272 in number, were cut longitudinally into halves and planted on May 2, 1917, one set of halves on the contaminated soil and the other set on similar, but uncontaminated soil a few yards away as a control. During growth 90 per cent of the plants on the diseased soil showed wilt, whereas none of the plants on the control soil showed any (pl. 6, A and B).



Wilt of potato plant in the field as a result of inoculation made by placing on the seed piece a pure culture of *Verticillium albo-atrum* on sweet clover stems before covering with soil, May 1, 1917. Plants photographed August 22. Variety, American Wonder

A.—Plant badly wilted, grown from inoculated healthy half tuber. When tested after harvest two tubers out of 10 yielded cultures of *Verticillium albo-atrum*

B.—Healthy control plant same age as A and grown from other half of same seed tuber. When tested after harvest none of the eight tubers produced showed any infection by *Verticillium albo-atrum*



Wilt of potato plant in the field as a result of infection by *Verticillium albo-atrum* coming from the overwintered fungus in old tops from the previous crop in the soil. Plants photographed August 22, 1917. Variety, Early Rose

A.—Plant nearly dead from wilt, grown from healthy half tuber on soil contaminated from previous crop. When cultured after harvest two tubers of the four produced showed infection by *Verticillium albo-atrum*

B.—Healthy control plant grown from other half of same tuber on soil which had not grown potatoes for five years. None of the 10 tubers produced showed any infection from *Verticillium albo-atrum* when cultured after harvest

When harvested and cultured, 40.2 per cent of the 647 tubers produced on the former plot gave *V. albo-atrum* and none of the 1,297 tubers from the latter gave this fungus in culture. It was from the yield of these two plots that the results used in Figure 1 were secured. The weight of the crop harvested from the diseased plot was only 32.5 per cent of that from the control plot. Not all of this reduction in yield is, however, to be attributed to Verticillium, as no doubt other factors entered in. For instance, *Fusarium radicicola* Wollenw. was isolated from 13.9 per cent of the tubers from the diseased plot and from only 4 per cent of those from the control plot. Only 29.8 per cent of the tubers from the former plot gave no growth of any organisms in the cultures, whereas 66.6 per cent of those from the latter gave no organism in culture. It is, of course, to be expected that where diseased potato tops were used to contaminate the soil a multitude of organisms besides the one under test would be introduced to affect the following crop and complicate the results.

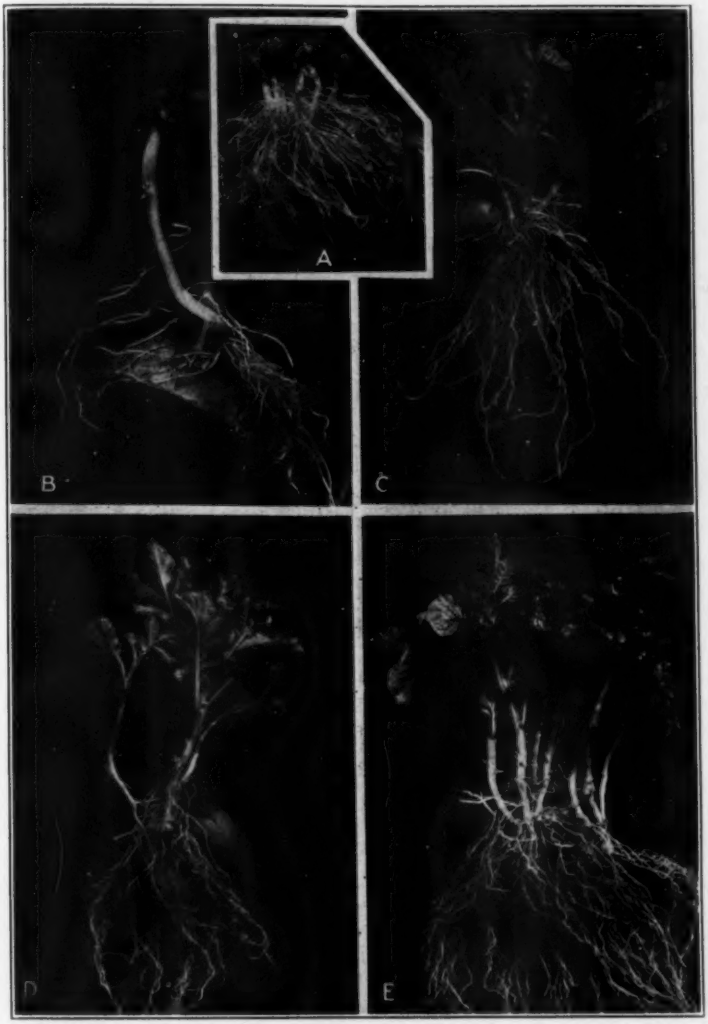
From the experiments just reported it is evident that infection does take place very readily from the soil and that the old diseased tops from previous crops may be a very important source of infection unless precautions are used to avoid this by crop rotations. One of the interesting questions that arise in this connection is how the organism gets into the plants from the soil.

MODE OF INFECTION FROM THE SOIL

From experiments conducted it is concluded that infection of potato plants by *Verticillium albo-atrum* from the soil takes place through the small roots and not through the seed piece as appears to be true at times with *Fusarium oxysporum* (*1*, *2*, *4*), or directly into the stem, although as to the latter no conclusive evidence is at hand to indicate that infection does not occur through the stem. The following experiments have a bearing on this matter.

On May 9, 1919, a series of wilt-free seed potatoes was planted in the field on well-rotated land and inoculated in the customary way by placing 27 to 34 day old cultures of *Verticillium albo-atrum* on sweet-clover stems on and near the seed pieces before they were covered with soil in the furrow. These sweet-clover stems were cut into pieces not over an inch in length and stirred into well water. About two tablespoonfuls of this infusion were used on each seed piece.

On June 10, when the plants were all coming up and were generally not over an inch high, 10 plants were dug, washed thoroughly, soaked three minutes in a 1 to 1,000 solution of mercuric chloride, rinsed through three vessels of sterile water, and cultured on potato glucose agar for the possible presence of *Verticillium albo-atrum* in different portions of the plants. The tissues cultured were macerated and incubated in poured Petri plates. All of the 10 seed pieces, of which 7 were cut and 3 whole, were entirely sound. The discolored tissue on the cut surface was not noticed to be deeper than 3 mm. Seventeen cultures made from this region or from the seed-tuber tissue just below the point of attachment to new stalks gave no growth of Verticillium. The 67 cultures made from the lower 2 inches of 23 stems gave no indication of infection in this region of the plants. Only two roots out of 68 tested gave *V. albo-atrum* in culture, and in each of these cases the fungus was growing directly out of portions



Mode of infection of potato plants in the field by *Verticillium albo-atrum*. Plants grown from healthy seed potatoes inoculated by placing cultures of the fungus on and near the seed pieces before they were covered with soil in the furrow at planting time, May 9, then dug up at intervals during growth and cultured for the presence of the organism in different tissues. Season of 1919. Variety, Up-to-Date

A.—The cultures made disclosed infection in one root but none in the stems of this plant. One other plant of the 10 tested at this time, June 10, gave similar results, the others revealing no infection

B.—Young plant from series dug June 10 with a portion of the inoculum carrying *V. albo-atrum* in a viable condition wedged between the bases of the two stems. The cultures made failed to reveal any infection in this plant

C.—One plant of 10 dug June 25 in which the cultures made showed infection in only one root. Infection was found in the stems only and not in the particular roots cultured in two other plants of this series

D.—One of five plants dug July 9 from which cultures of *V. albo-atrum* were secured from only one stalk and one attached root. A total of three of the five plants tested on this date showed the presence of infection in some portion

E.—Another one of the plants dug July 9 in which infection was found in one stalk and also in one stolon 2 inches long. This is the earliest date in the season at which the presence of *Verticillium albo-atrum* has been observed in a stolon.

of the root tissue which was perfectly sound and not in any way affected by rot. These roots were 4 inches and 2½ inches long, respectively, and came from two separate plants, one of which is illustrated in Plate 7, A.

One plant of this series (pl. 7, B) when dug had a piece of the sweet-clover stem from the inoculum wedged between the bases of the two stems of the young plants. The 14 cultures made from this plant gave no evidence of infection either in the roots or in the stems. However, when the piece of clover stem was treated in mercuric chloride solution for three minutes, rinsed through three changes of sterile water, and cultured, Verticillium was readily isolated, showing that the inoculum was still virulent. The fact that the stems of these 10 potato plants gave no evidence of infection even when the inoculum was very favorably situated may indicate that infection does not take place directly in the stems. At any rate the evidence here is that at this stage of development the fungus was present in only a few roots and not in other parts of the plants.

On June 25, 10 additional plants from this series were dug and cultured as before. The 26 cultures made from the seed pieces, 137 from roots, 87 from stems, and 42 from stolons ranging from 0.5 to 4 inches in length gave evidence of infection in three of the plants. In one plant (pl. 7, C) only one root 8 inches long out of 18 cultured gave Verticillium. The 9 stem, 6 stolon, and 3 seed-piece cultures from this plant all gave negative results. One other of these three plants gave positive results in two stem cultures at 0.5 inch from the lower end and at the soil line, but not from two other stem cultures or from the 12 root, 5 stolon and 3 seed-piece cultures. The other of the three plants showing infection gave only one positive culture, and that from a section of the stem near the soil line. Negative results were obtained from the other 3-stem cultures made from nearer the base, the 6 root, 1 stolon, and 3 seed-piece cultures. The evidence here, then, shows that at this stage infection was present in the roots and stems but not in the stolons. Two plants revealed the presence of the fungus in the stems, though the particular roots tested from these plants showed no infection.

Finally, on July 9, five more plants from the same inoculation series were cultured as previously, except that the mercuric-chloride solution for treating the plants was used at 1 to 500 for five minutes. Three of these plants showed infection by Verticillium, one (pl. 7, D) in an 8-inch root and in all portions of the stem underground except the lowest one-eighth of an inch, another in one section of the stem only, and the third (pl. 7, E) in the lowest one-eighth of an inch of the stem and in one stolon 2 inches long. This is the earliest date in the season at which the presence of *V. albo-atrum* has been demonstrated in a stolon. Infection, then, at two months after planting was present in roots, stems, and stolons. The cultures from the three sets of plants seem to show approximately that infection took place first in the roots, then in the stems, and finally in the stolons. At least, infection was first found in the plants in the order named.

That infection can and does take place through the roots is further indicated by some additional tests performed. In 1921 some inoculations were made by placing the fungus growing on clover stems in

the bottom of some narrow trenches dug to different depths below the seed pieces, filling the trench with the soil thrown out, and then planting the seed pieces at the usual depth. Inoculations, using 10 hills in each case, were made with the fungus 1, 2, 3, 6, and 9 inches below the seed pieces. The controls in this case consisted of inoculations made in the usual way by placing the inoculum in contact with the seed pieces before covering. At harvest time the fungus was apparently fruiting alike on all the stalks. When cultured the tubers harvested showed infection as follows: "Contact," 18.2 per cent; 1 inch, 18.2 per cent; 2 inches, 15.8 per cent; 3 inches, 27.8 per cent; 6 inches, 27.3 per cent; and 9 inches, 23.1 per cent. Although the variations between these lots may not be significant, it is interesting to note that at 3, 6, and 9 inches infection in the tubers was uniformly higher than in the other cases.

A similar test was performed in 1922, in which the inoculum was placed in contact with the seed pieces and at depths of 3, 6, and 9 inches below the seed pieces, one lot being left without inoculation. Ten plants were used in each lot. When cultured after harvest the tubers from the uninoculated control plants showed no wilt infection, those from the "contact" inoculated plants gave 28.5 per cent infection, those at 3 inches 27 per cent, at 6 inches 35.5 per cent, and at 9 inches 57.5 per cent. Here also the highest infection in the tubers occurred where the inoculum had been placed at the greatest depth in the soil. A possible explanation of this is that since both seasons in which the tests were made were relatively dry the soil moisture may have been greater and more favorable to the growth and to the infection of the plants where the deeper trenches were dug below the seed pieces. Infection of the plants where the inoculum was placed 9 inches below the seed must surely have come about only when the roots grew down and came in contact with the organism. It does not seem likely that the fungus could grow through the soil to any appreciable extent.

When the work on the effect of roguing for checking the spread of wilt was under way, the question arose as to how far away from the seed piece the fungus could be located and still cause infection of the plant. Some experiments to throw light on this point are presented here, for they also tend to show that infection enters the plant through the root system. One test was performed in 1922, in which the inoculum, consisting of the usual sweet-clover-stem cultures of the organism, was placed in contact with the seed pieces and in the furrow at different distances from the seed pieces, namely, at 15, 30, 45, and 60 inches. So little wilt developed in the contact-inoculated series—the tubers harvested showed only 11.9 per cent infection when cultured, owing probably to the dry season—that dependence could not be placed on the results. Consequently a somewhat similar test was made the following year.

In the 1923 experiments the plants were spaced at suitable distances apart, and the inoculum was placed in the bottom of trenches dug about 8 inches deep on the four sides of and extending about half way around each hill treated by this method. Some plants were inoculated by placing the inoculum in contact with the seed piece and others were left uninoculated as controls. The plot was kept free of weeds by hoeing and no cultivation was done after

planting. After harvest the tubers were cultured by the sweet-clover-stem method for the presence of *Verticillium albo-atrum*.

Definite wilt of some of the plants even in the "45-inch plot" but none in the "60-inch plot" was noted during growth. However, the tuber cultures and not the appearance of the plants are relied on chiefly for judging the extent of infection. The results (Table IV) show the highest infection and the greatest reduction in yields in the plants inoculated by placing the inoculum at a distance of 15 inches from the seed pieces. It is probably only accidental that this lot showed more disease than those inoculated through contact. However, the significant thing about the test is the evidence of considerable infection in the 30 and 45 inch plots.

TABLE IV.—*Results obtained from inoculations made with Verticillium albo-atrum by placing the inoculum in the soil at different distances away from the seed piece. Variety, Up-to-Date, season of 1923, Corvallis, Oreg.*

Location of inoculum	Number of hills	Average yield per hill		Number of hills yielding infected tubers	Tubers produced		
		Number of tubers	Weight in ounces		Total number	Number infected by V. albo-atrum	Per cent infected
In contact with seed piece	7	9.3	37.6	4	65	13	20.0
15 inches from seed piece	13	6.7	19.9	10	87	31	35.6
30 inches from seed piece	14	7.6	32.1	8	103	16	15.5
45 inches from seed piece	10	11.2	53.8	7	109	26	23.9
60 inches from seed piece	8	7.3	38.6	1	56	1	1.8
Uninoculated control	14	8.0	48.0	1	111	6	5.4

Early in August a little digging in the top 8 inches of soil showed that the roots of the plants were commonly extending out 30 inches but not as far as 45 inches from the base of the plants. Late in September, however, the roots of some of the plants had grown out to a distance of 45 inches. None were ever found extending as far as 60 inches. This is a much wider distribution of surface roots than was noted by Weaver, Jean, and Crist (7) in their study of the root development of potatoes in Nebraska where the maximum lateral spread found was about 26 inches. In the present case there was no competition between the root systems of the different plants spaced so far apart, and the roots were not hindered in their development by cultivation. Their distribution in the surface inches of soil under such conditions is naturally more extensive than would be the case with plants spaced close together in the row and cultivated in the usual way.

It is reasonably certain that infection of these plants took place when the roots had grown out into the region where the inoculum had been deposited. Naturally, the 15-inch lot would show wilt before those where the inoculum had been put farther away. That this occurred to a striking degree is indicated by the illustrations of a few of the plants photographed September 28 in the 15, 30, and 45 inch plots (pl. 3, C, D, E). The 15-inch inoculations caused earlier death of the plants and the production of smaller yields than occurred in the other two cases. Some of the difference in size of

yields would, however, be due to the disparity in the amount of space the plants were allotted in the different plots.

The 60-inch inoculations may be considered to have given negative results, since none of the plants showed wilt symptoms during growth and only one tuber out of 56 produced in the eight hills indicated infection when cultured. One of the 14 uninoculated control plants was affected by wilt, and six of the 17 tubers produced by it gave the wilt fungus in culture. This infection may have come from a chance contamination in the soil, since some rotation plots were being conducted a few rods away.

From the experiments reported it appears reasonably certain that infection from the soil occurs largely if not entirely through the root system. Also infection may apparently take place when the fungus is at a distance of 45 inches from the seed piece provided the roots are not prevented from growing to this distance. Evidently the limit of distance at which infection can take place in the soil is determined by the limits of growth of the root systems themselves. Since the soil is so permeated by the root systems of potato plants the seriousness of the occurrence of any infective material in the soil becomes very striking. For the chances are exceptionally good that any infective material in the soil would be picked up by the roots and transmitted to new crops through the seed potatoes. This fact only serves to emphasize the necessity for the use of rotation cycles of sufficient length to eliminate all traces of this wilt fungus from the soil between crops of potatoes, and particularly those to be used for seed purposes.

It is apparent from the experiments reported that infection of potato plants with *Verticillium albo-atrum* is very readily secured by artificial contamination of the soil. This is in considerable contrast to the usual result when attempts are made to infect potato plants with *Fusarium oxysporum*. One parallel inoculation experiment conducted by the writer using identical methods with these two organisms, namely, placing the inoculum consisting of cultures grown on sweet-clover stems on the seed piece before covering with soil, gave with the former 99 per cent wilt and 49 per cent infection of the tubers and with the latter no wilt symptoms in the plants and only 6 per cent infection of the tubers.

MacMillan (*4, p. 283*) reported having to resort to the method of placing the inoculum in wells in the cut seed piece in order to secure a successful lot of infections of potato plants with *F. oxyporum* in Colorado. Bisby (*1, p. 12*) had little success in securing infection of potato plants with this fungus and concluded that it is not vigorously parasitic to actively growing potato plants. Where entrance of the fungus into the plants was secured, it apparently took place largely by way of the seed piece. Goss (*2*) failed to secure very successful infections by any methods used, and states that infection from the soil as studied in Michigan and Nebraska was usually through the roots rather than through the seed piece. Other workers have reported rather low percentages of infection following inoculations with *F. oxysporum*. Evidently *V. albo-atrum* gains entrance to potato plants much more readily and appears also to be uniformly more actively parasitic to potatoes than does *F. oxysporum*.

LENGTH OF ROTATION NECESSARY FOR CONTROL

LONGEVITY OF FUNGUS AS INDICATED BY CULTURES

To secure some knowledge of the longevity of *Verticillium albo-atrum* in old potato stalks in the soil a test was begun in the fall of 1916. Stalks from wilted hills on which the fungus could be seen growing on the surface were collected September 28, stored in an unheated storeroom until November 10, and then scattered on the surface of the soil and buried at depths of 1, 3, and 6 inches in a plot exposed to natural outdoor weather conditions. Examinations made December 11 showed that the fungus was fruiting freely at all depths in the soil and on the surface. The conidia were viable Examinations and cultures from this material were made again on March 1, 1917. When collected, no growth of *V. albo-atrum* was evident on the surface of the stalks on top of the ground. However, at all depths in the soil the grey masses of the Verticillium conidiophores were evident. Plate-culture tests showed that the fungus was viable at all stations, though only one stem in the four tested from the surface gave the fungus in culture, whereas all the buried stems tested gave positive results.

On May 7, 1917, somewhat past the best planting date for the early potatoes in this region, stalks from the overwintering test were again dug and examined. The fungus was not found fruiting on any of the stalks which had passed the winter on the surface of the ground. When tested, however, 1 stalk in 4 gave the fungus in culture. Also 1 stalk (pl. 1, B) from this lot, after being buried under a few inches of soil out of doors for about 10 days, gave an abundant production of conidiophores and conidia of *Verticillium albo-atrum*. Of the stalks that passed the winter buried in the soil at depths of 1, 3, and 6 inches, 2 stalks out of 3 tested, 1 out of 3, and 3 out of 4, respectively, gave an abundance of growth of *V. albo-atrum* in culture. On some of these stalks the fungus was fruiting when they were dug from the soil. One of the positive cultures obtained from a stalk at the 3-inch depth is pictured in Plate 1, C. This Petri plate was made with potato glucose agar to which was added at the time of pouring 3 drops of 10 per cent lactic acid. It contains approximately 200 colonies of *V. albo-atrum* and only a few colonies of other organisms. Some of the original material which had been kept dry in a packet in the laboratory since the tops were collected on September 28, 1916, was now tested, and all of the stalks failed to give growth of this fungus. A year later, on May 20, 1918, another examination was made to determine the viability of the fungus in this material. At that time only small pieces of the original potato stalks could be found at any depth in the soil, and this only with difficulty. A few pieces were still present on top of the ground; no tissue was found at 1 inch; and only a little, very greatly disintegrated, at 3 to 6 inches. Samples of this tissue, including portions of 14 stalks from the surface of the ground, 6 stalks at 3 inches, and 3 stalks at 6 inches, were macerated and incubated in poured Petri plates, potato glucose agar and 3 drops of lactic acid being used in each plate. All results were negative. Microscopic mounts made from the remains of 1 stalk at 6 inches showed some old conidiophores of Verticillium. These were shriveled and appeared to be

dead. Some sclerotiumlike bodies and masses of heavy mycelium quite similar in form and color to the mycelium of *Verticillium albo-atrum* commonly present in disintegrating potato stalks were found which appeared unshrunken and looked to be alive. However, hanging drops and poured plates made from this material failed to give any indication of growth of *V. albo-atrum*.

These results, then, show that this wilt fungus lives very readily in old potato tops in the soil from harvest in the fall through one winter to planting time the next season, but indicate that it is not able to live thus through two winters to the second planting season. From this test alone it would appear that the planting of soil with a nonsusceptible crop for one year would be sufficient to rid it of contamination by *Verticillium albo-atrum*, provided the matter were not complicated by the holding over of the organism by volunteer potato plants. Additional tests including field-planting trials have, however, been necessary before any definite conclusions could be drawn on the length of rotation required for control of the disease.

Results from Rotation Tests

A series of field trials was planned and carried out in an effort to determine the results that could be secured in avoiding this disease by crop rotations of different length. No diseased field available was suitable for the tests, so a selected piece of ground containing one and a half acres was laid out into 16 one-twentieth-acre plots for the purpose in 1919. It was thought that a four-year rotation would be sufficient for freeing the soil of infection, so provision was made for determining the influence of one, two, three, and four year rotations for this purpose. The tests were run in duplicate. In one series (Table V) the plots (Nos. 1 to 4) were all contaminated one year and the test crops were grown in different years. In the other series the plots (Nos. 9 to 12) were contaminated in different years and the test crops were grown in the same year. This was done because variations in climatic conditions in different seasons might be expected to have an influence on the extent to which the fungus would be held over in the soil and the amount of disease that would develop in the test crop grown for determining the extent of occurrence of the fungus in the soil. For each test plot there was a corresponding control plot on which the crops were grown in the same order and at the same time as on the test plot, the only difference between the plots being the introduction of the disease in the beginning in the one case and not in the other.

In order to have the diseased soil plots heavily contaminated with the wilt organism a crop of potatoes was planted and inoculated at planting time in the usual way with pure cultures of the fungus on clover stems grown for the purpose in quantity in quart jars. Uninoculated potatoes were grown on the corresponding control plots the same year. Then following this at different intervals, as indicated in Table V, a crop of the most nearly wilt-free seed potatoes available was planted on the plots to test for the extent of infection from the soil. This crop for convenience is termed the test crop. The Up-to-Date variety was used throughout these trials.

TABLE V.—*Results obtained from tests conducted to determine the influence of rotation cycles of different length on the amount of Verticillium infection secured from the soil. Variety, Up-to-Date, seasons of 1919 to 1923, Corvallis, Oreg.*

Length of rotation in years[a]	Diseased soil plots					Control plots		
	Plot No.	Year inoculated potatoes were grown for contaminating soil[b]	Year test potatoes were grown	Tubers from test crop cultured for wilt infection		Plot No.	Tubers from test crop cultured for wilt infection	
				Number	Per cent infected by V. albo-atrum		Number	Per cent infected by V. albo-atrum
1	1	1919	1920	380	34.2	5	682	18.7
2	2	1919	1921	287	22.3	6	190	11.1
3	3	1919	1922	603	3.6	7	329	.6
4	4	1919	1923	630	3.8	8	310	.3
1	9	1922	1923	327	24.5	13	319	.9
2	10	1921	1923	302	20.5	14	307	1.3
3	11	1920	1923	302	1.3	15	319	.6
4	12	1919	1923	311	.3	16	323	1.2

[a] The crops used in the rotations were as follows: 1-year rotation, potatoes; 2-year rotation, grain and potatoes; 3-year rotation, grain, clover, and potatoes; and 4-year rotation, grain, clover, clover, and potatoes.
[b] Uninoculated potatoes were grown on the corresponding control plot the same year.

The crops commonly used in rotation with potatoes vary with each locality and almost with each farm. In the opinion of the department of farm management of the Oregon Agricultural College the best basic crop rotation for use in western Oregon is grain, clover seed, clover hay, and a cultivated crop. A variety of products including potatoes is used as the cultivated crop. In these experimental plots use was made of only grain and clover for the rotation crops, as shown in the table. The three-year rotation as used here, namely grain, clover, and potatoes, is one that is commonly used in western Oregon.

A representative number of tubers, usually about 300 from each of the test crops, were cultured in the laboratory in the usual way for determining the extent of occurrence of *Verticillium albo-atrum* therein. The percentage of tubers infected rather than the appearance of the plants during the growing season is used as the index for judging the extent of infection from the soil because it is the more reliable method.

In both sets of tests the one and two-year rotation plots gave a considerable amount of the wilt disease owing to infection from the soil, but the three and four year rotations can be considered to have given practically complete control (fig. 5). It is true that plots 3 and 4, bearing a three and four year rotation, do show a noticeable amount of wilt, 3.6 and 3.8 per cent, respectively. It is believed that this infection resulted from some uncontrolled factors in carrying out the tests rather than from the holding over of the fungus in the soil for this length of time.

Unfortunately, in conducting the tests at least four factors were not as completely under control as was desired: (1) The plots were under flood water twice during the five years in which the experiment was being run, affording an opportunity for the transfer of infective

material from one plot to another. (2) Once after plowing the plots in a sequence that would avoid transfer of undesirable material from plot to plot they were by error disked crosswise under conditions that made possible the carrying of contamination from one plot to another, even though separated by two-row and four-row alleyways. (3) The seed potatoes used for growing the test crops were not always completely wilt-free and occasionally, no doubt, carried some infection into the plots. This accounts for some of the disease in the test crops. (4) The seed potatoes used on the control plots the first year, that is in plots Nos. 5 to 8 in 1919, when the diseased-soil plots were being contaminated, contained some wilt which gave rise to diseased plants. This material held the fungus alive in the soil

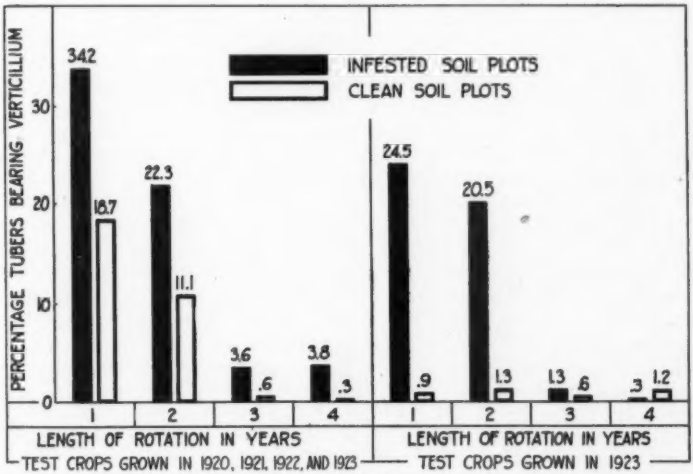


FIG. 5.—Graph showing the influence of the length of the rotation cycle on the amount of infection by *Verticillium albo-atrum* secured from the soil

over winter and undoubtedly was the cause of the appreciable infection, 18.7 per cent in plot 5 in 1920, and 11.1 per cent in plot 6 in 1921. However, since plots 5 to 8 were originally planted with the same seed, and since the test crops grown on plots 7 and 8 representing three and four year rotations showed so little disease, 0.6 and 0.3 per cent, respectively, as compared to plots 5 and 6 representing one and two year rotations, this series of plots really furnishes an additional illustration of the effectiveness of the three and four year rotations in eliminating infection from the soil.

Considering the fact that the rotation tests were conducted without all factors being fully under control and that plots Nos. 1 to 4, 5 to 8, and 9 to 12 furnish three separate tests of the effectiveness of rotations—the three-year rotation tests having given 3.6, 0.6, and 1.3, and the four-year rotation tests 3.8, 0.3, and 0.3 per cent disease, respectively—it seems reasonable to conclude that the three and four year rotations are practically completely effective in avoiding Verticillium wilt infection from the soil.

Interest in these results is perhaps increased when it is recalled that there are few definite figures available dealing with the influence of crop rotations on the elimination of potato diseases from the soil. This situation was strikingly brought to mind by a statement of Shapovalov and Link (*6, p. 8*) in a general discussion of potato diseases that "no case is known in which parasitic organisms have been completely eradicated from the soil by means of crop rotation, but a substantial decrease in the severity of infection may be expected in some cases." In Verticillium wilt, then, we appear possibly to have the unique illustration of a potato disease which can be completely controlled, in so far as the soil is concerned, by means of crop rotation.

SUSCEPTIBILITY OF VARIETIES TESTED

No attempt has been made to conduct a comprehensive test of the comparative susceptibility of different varieties of potatoes to Verticillium wilt. However, during the progress of the investigation some information has been collected to which brief reference will be made. In 1917 two infection experiments were performed in which eight potato varieties were used. In one test the seed was planted in soil contaminated from the previous crop and in the other pure-culture inoculations were made, using the fungus growing on sweet-clover stems. As judged by the percentage of infected tubers produced, each variety performed quite similarly in the two tests, so only an average of the two is given here. The varieties used and the percentage of infected tubers produced by each are as follows: Rural New Yorker, 59.1; Up-to-Date, 53.8; Early Rose, 52.3; American Wonder, 43.4; Gold Coin, 39.4; Burbank, 37.5; Uncle Sam, 35.8; and Irish Cobbler, 25 per cent. These figures may indicate a difference in the varieties, but this difference is no doubt more apparent than real. The Irish Cobbler plants wilted as readily as did the others, though only one-fourth of the tubers produced were wilt infected, as compared to one-half of the tubers in the case of some of the other varieties. The Irish Cobbler was the first variety to mature, and it is thought that the earliness of maturity alone was responsible for the small number of infected tubers produced. Other varieties which were affected with Verticillium wilt but were not included in any comparative trial are Garnet Chili, Netted Gem, Pride of Multnomah, and Earliest of All. Ot these additional varieties observed there is no outstanding difference evident in their reaction to the disease. Consequently none of the 12 varieties noted particularly appear to possess any noticeable resistance to Verticillium wilt.

SUMMARY

Additional evidence has been obtained which confirms the conclusions that the presence or absence of *Verticillium albo-atrum* in potato tubers can not be reliably determined from a mere inspection of the tubers. The separation of tubers with discolored stem ends from those that are nondiscolored is of doubtful value for Verticillium wilt control. Nor can the discarding of stem ends and the planting of only eye ends from suspected tubers be recommended as a specific measure of control, for the eye-end pieces at times give as much

disease as the stem-end pieces. When the fungus is present in seed potatoes it is readily transmitted to the yields.

Verticillium albo-atrum spreads from one plant to another in the row during the growing season. Infection of the second plant away from the originally wilted plant in the same row is not uncommon, and noticeable spread to the third plant distant has been demonstrated. This spread of the fungus from one plant to another apparently takes place underground through contact of the root systems.

Many potato plants contaminated from the adjoining wilt-diseased plants do not show visible wilt symptoms in the field, probably because of late infection. Consequently all wilt-infected plants can not be detected by field inspections. For this reason effective control of the disease can not be secured by the roguing of only the visibly wilt-diseased plants. For effective control, use must be made of the three-plant method of roguing; that is, the noticeably wilt-diseased plant and the next adjoining healthy appearing plant on either side in the same row should be removed at the same time. In one test where the three-plant method of roguing was used, a reduction of from slightly more than 22 per cent visible wilt infection in the plants to 3.2 per cent infection in the tubers was obtained in one season's time, whereas the roguing of only the noticeably wilt-affected plants in an adjoining plot reduced the infection to only 8.4 per cent in the tubers remaining in the plot. Where Verticillium wilt is present, the three-plant method of roguing should be used for all potatoes grown for seed purposes.

Infection of potato plants from the fungus in the soil occurs readily and appears to take place through the roots. Infection occurred freely when the inoculum was placed at a depth of 9 inches below the seed piece and also at distances of 30 and 45 inches horizontally from the seed piece. Evidently the limit of distance at which infection can take place in the soil is determined by the limits of growth of the root systems themselves.

Viability tests conducted show that *Verticillium albo-atrum* lives very readily in old potato tops in the soil from harvest in the fall through one winter to planting time the next season, but indicate that it is not able to live thus through two winters to the second planting season. From this it would appear that the planting of soil with a nonsusceptible crop for one year would be sufficient to rid it of contamination by this fungus. However, field rotation trials showed that a two-year rotation was ineffective for avoiding infection from the soil. On the other hand, three and four year rotations apparently can be considered completely effective in eliminating the fungus from the soil.

Of the 12 potato varieties particularly noted, none seems to possess any appreciable resistance to Verticillium wilt.

LITERATURE CITED

(1) Bisby, G. R.
1919. STUDIES ON FUSARIUM DISEASES OF POTATOES AND TRUCK CROPS IN MINNESOTA. Minn. Agr. Exp. Sta. Bul. 181, 58 p., illus.

(2) Goss, R. W.
1923. RELATION OF ENVIRONMENT AND OTHER FACTORS TO POTATO WILT CAUSED BY FUSARIUM OXYSPORUM. Nebr. Agr. Exp. Sta. Research Bul. 23, 84 p., illus.

(3) McKay, M. B.
1921. TRANSMISSION OF SOME WILT DISEASES IN SEED POTATOES. Jour. Agr. Research 21: 821–848, illus.

(4) MacMillan, H. G.
1919. FUSARIUM-BLIGHT OF POTATOES UNDER IRRIGATION. Jour. Agr. Research 16: 279–304, illus.

(5) Pethybridge, G. H.
1916. THE VERTICILLIUM DISEASE OF THE POTATO. Sci. Proc. Roy. Dublin Soc. (n. s.) 15: 63–92, illus.

(6) Shapovalov, M., and Link, G. K. K.
1924. CONTROL OF POTATO-TUBER DISEASES. U. S. Dept. Agr. Farmers' Bul. 1367, 38 p., illus.

(7) Weaver, J. E., Jean, F. C., and Crist, J. W.
1922. DEVELOPMENT AND ACTIVITIES OF ROOTS OF CROP PLANTS. A STUDY IN CROP ECOLOGY. 117 p., illus. Washington, D. C. (Carnegie Inst. Wash., Pub. 316.)

PHYTOPHTHORA BUD ROT OF COCONUT PALMS IN PORTO RICO[1]

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INTRODUCTION

The coconut palm (*Cocos nucifera*) is one of the most widely distributed of tropical plants, its habitat encircling the earth as an equatorial belt about 3,000 miles wide. It origin is obscure. Early European explorers noted the palms flourishing on most of the coasts where they grow to-day. It is not unlikely that some of the nuts, falling from palms growing along shore, were transported by ocean currents to distant beaches, where they became embedded in the sand and germinated.

The coconut palm thrives in all parts of Porto Rico except in the semiarid southwestern section. It is largely confined to sandy beaches which are unsuitable for other crops, and makes excellent growth in such locations provided there is sufficient rainfall. Occasionally it does well in the interior. The coconut palm is easily cultivated. The nuts, or two-year old seedlings, are planted in rows about 35 feet apart, and the planted area is weeded until the palms are 3 or 4 years old. After this the area is given an occasional cleaning. The plants begin to bear when they are 8 to 10 years old, and produce crops regularly for years. The profits gradually decrease with the advancing age of the palm, however, due to decrease in size of the nuts and increase in the cost of picking.

The coconut palm does not hold the high position as a source of food and shelter in Porto Rico that it occupies in some islands of the East, but it does hold an important place. Porto Rico annually exports about $500,000 worth of ripe nuts, and both ripe and green nuts netting an equal sum are marketed locally each year. Fallen coconut leaves are used by the poor to thatch their dwellings and to build fences. The most serious disease of the coconut palm is bud rot.[2]

HISTORY AND DISTRIBUTION OF BUD ROT

The earliest record known to the writer of the occurrence of coconut bud rot places it at Grand Cayman, an island of the British West Indies just south of Cuba, in 1834 (*8, p. 3–4*).[3] Since that time the disease has been reported from nearly every coconut-producing region of the world. Malaya and the Dutch East Indies are probably the largest territories in which the disease is unknown, according to Sharples and Lambourne (*17*) and Hartley and Rands (*10*). The distribution of the disease through both hemispheres, and the history of attempts to demonstrate the causal organism as late as the year 1910 are well discussed by Johnston (*11*), who attributed the bud rot found in Cuba to an organism which was "practically identical with *Bacillus coli* (Escherich) Migula."

[1] Received for publication Feb. 20, 1925; issued February, 1926.
[2] The name by which the disease is known throughout English-speaking countries.
[3] Reference is made by number (italic) to "Literature cited," p. 497.

In India, in 1910, Butler (*4*) described a bud rot of palmyra and coconut palms, isolated a fungus which he named *Pythium palmivorum* Butler,[4] later placing it in the genus Phytopthora (*5*), and reproduced the disease by inoculation. He did not use pure cultures for his inoculations, but, in 1914, Shaw and Sundararaman (*18*) obtained typical cases of bud rot in 75 per cent of the inoculated palms. No statement is made as to the number of palms inoculated, nor is the method used described. In 1923, McRae (*12*) published the results of successful inoculations of both *Borassus flabellifer* Linn. and *Cocos nucifera* Linn. with *Phytophthora palmivora*.

Ashby (*1*), in Jamaica, in 1920, reported two types of coconut bud rot; one he believed to be of bacterial origin and identical with the Cuban trouble; from the other type he obtained a fungus which was identified by Butler as *Phytophthora palmivora* Butler. Inoculations failed to reproduce the disease, though he succeeded in producing rows of spots on the unfolding leaves and a decayed spot on a leaf base from which the fungus was reisolated.

In 1919, Reinking (*14*) produced bud rot in the Philippines, using pure cultures of *Phytophthora faberi* Maublanc, isolated from rotted cacao pods for inoculation of wounded seedlings and unwounded trees. Further experiments (*15*) revealed that the Phytophthora strains, isolated from cases of coconut bud rot and from rotted cacao pods, were identical morphologically and physiologically.

Bud rot has recently been reported from southern Florida by Burger (*3*). A culture of *Phytophthora faberi* Maublanc, isolated from rotted buds, has been received from G. F. Weber.

Bud rot, or a similar disease, is causing considerable losses in Costa Rica, Mexico, British Honduras, Trinidad, British Guiana, Haiti, and Santo Domingo. There are few reports offering conclusive evidence as to the nature of the disease in these countries.

BUD ROT IN PORTO RICO

In the past the coconut palms of Porto Rico have been remarkably free from disease. Some young seedlings have been killed each year by the rhinocerous bettle (*Strataegus* sp.), and an occasional older palm has succumbed to the ravages of a small beetle (*Xyleborus* sp.) that bores into the trunk.

Johnston visited Porto Rico in 1907 and again in 1910–11 to search for bud rot along the coasts. In his report (*11*) he states: "In 1910 the writer saw five or six trees on the coast between Anasco and Corsica which had very much the general appearance of bud rot. Closer examination in the present year (1911) showed the diseased tissues to be somewhat similar to that of bud rot but not typical."

In 1910, Fawcett (*7, 1910*) noted the occurrence of a bud rot in "some of the plantations along the west coast," in connection with which discovery he says (*7, 1911*) that "There can be no doubt that some of the trees apparently suffering from this bud rot have really been injured by lighting. In every case studied bacteria have

[4] Since this paper was submitted for publication, a paper by Butler, E. J., BUD ROT OF COCONUT AND OTHER PALMS, reprinted from the Report of the Imperial Botanical Conference, London, July, 1924, has been received. In this paper Butler has cleared up an uncertainty regarding *Phytophthora palmivora* Butler. His statement regarding the presence of oospores in the species is as follows: "It is true that in my 1907 paper I stated that oospores were formed, but these were subsequently found to be chlamydospores or resting conidia. . . ." The fungus under discussion in this present paper must be referred to *Phytophthora palmivora* Butler, as this name has priority over *Phytophthora faberi* Maublanc.

been isolated from the innermost diseased tissue, but uniformity in the results of such studies has not been obtained." More recently (*7, 1912*) he records having produced a "cut" appearance of the leaves by inoculation with *Thielaviopsis ethaceticus* (*paradoxa*), and writes that "The trees with 'cut' leaves were said by the owners of the grove to invariably succumb to bud rot after a while."

In 1924, the writer (*19, 20*) published brief notices of the appearance of the disease and a description of the symptoms.

In September, 1923, a coconut grove of 440 palms on the beach near Mayaguez was found to contain 62 bud-rotted palms. Observation indicated the infectious nature of the disease, the diseased palms usually occurring in fairly well-defined groups. According to the owner of the grove, about a dozen diseased palms had been noticed three years previously, and infection had steadily advanced until about 14 per cent of the grove was destroyed. During the winter of 1923–24, a survey was made of the west-coast coconut district to determine the incidence of the disease. In this district the palms are grown on a narrow fringe of shore extending only a few hundred yards back from the sea. Nearly every plantation between Mayaguez and Rincon was infected. The disease had reached alarming proportions at the mouth of the Añasco River, where 25 per cent of a 20-acre plantation had succumbed. Slightly more than 800 cases of active disease were recorded. The term "active disease" is used to exclude stumps, which were numerous in bud-rot-infected groves. Reduction of the palms to stumps likely had been caused by bud rot, and to include the stumps would nearly double the number of observed cases. The map (fig. 1) shows the location of the diseased groves. Each dot represents 10 cases of bud rot.

SYMPTOMS

The first visible symptom of bud rot is the death of the young emerging leaf in the center of the central column of young, green, erect leaves. The dead leaf is yellowish-brown in color, and resembles dead leaves in which chlorophyll failed to develop. It may easily be pulled from the inclosing sheath. Examination of the bud at this stage shows that the disease is converting the young leaf and the primordial tissue of the single growing point into a soft, watery, malodorous mass. The disease may extend into the more mature hardened tissue of the trunk, where the limit of its advance is marked by a narrow reddish-brown zone. Usually, the young swords and flowering racemes are invaded and rotted before they attain a foot in length. Within one to four weeks the young leaves surrounding the apex of the trunk begin to rot at the base, turn brown, and, with the heart leaf, are soon broken over by the wind and hang tips downward (fig. 2). They finally fall from the central column, leaving the palm conspicuous by the absence of a crown of young, erect leaves (fig. 3). The older leaves may retain their normal, nearly horizontal position and green color for many months, after which they turn yellow and gradually fall away until defoliation is complete (fig. 4). The course of the disease is invariable. The first dead leaf denotes the decay of the growing point, and the coconut palm does not produce a second one.

Examination of leaf bases and sheaths that are removed from the diseased bud usually discloses the presence of a dark-brown decayed spot 1 to 2 inches in diameter near the base of one of the inner leaves (fig. 5). The spotted tissue may be either fibrous or soft and

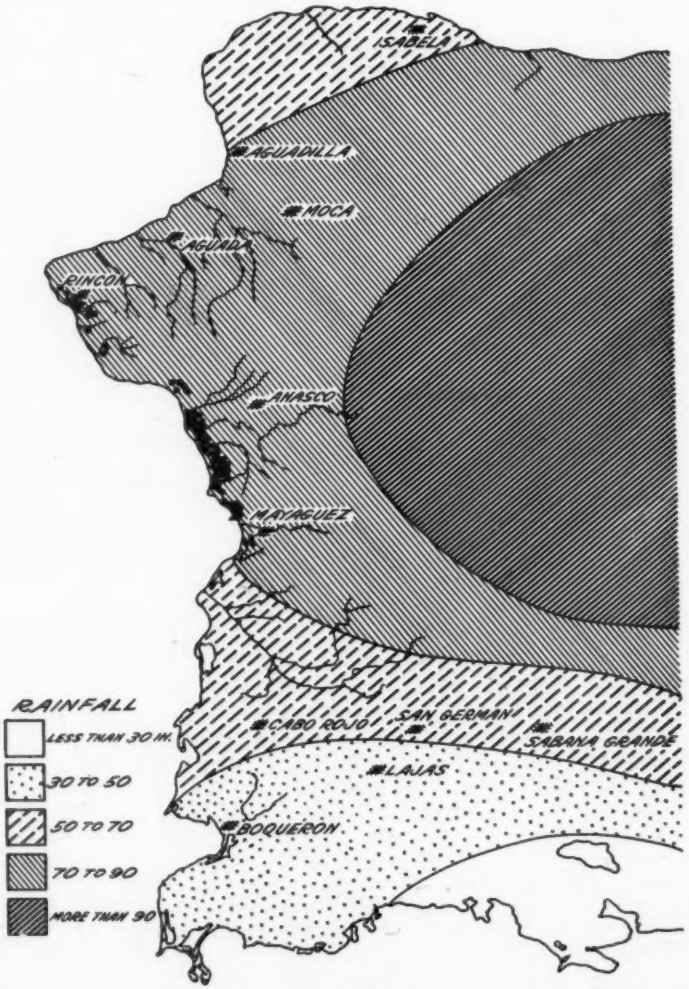

FIG. 1.—Map of western end of Porto Rico, showing distribution of rainfall, and localities where coconut bud rot is prevalent. (Each dot represents 10 infected palms)

watery, depending upon the stage of maturity reached by the leaf. On the removal of the spotted leaf, the underlying leaf base will be found to be similarly affected. The infection may spread through the intervening sheaths to the growing point, increasing in activity as the tissue encountered becomes soft and watery (figs. 6 and 7).

An occasional case reveals vertical penetration, as was observed by Reinking (*15*), who did not find lateral penetration of the leaf base such as commonly occurs in Porto Rico, and in Jamaica and Malabar, according to Ashby (*1*) and to Shaw and Sundararaman (*18*). As the growing point is destroyed the disease invades the apex of the

FIG. 2.—Coconut palm naturally infected by bud rot. Note absence of central column of erect leaves as compared with the palm to the right. Pendant young leaves may be noted on left side of the palm, above the old leaves, which nearly touch the ground

trunk until it reaches the hardened woody tissue. A cavity filled with a yellowish or grayish semiliquid putrescent mass is found in the apex. The soft pith of the trunk is destroyed to a depth of about a foot; the tough vascular tissues resist decay. There is no discoloration or other evidence of disease in the trunk below the cavity.

The writer has observed no cases of nut infection in the field. Occasionally racemes bearing nuts with a diameter of 1 to 2 inches blacken and fall from the bud-rotted palm, but this is due to decay at the base of the supporting raceme (fig. 8). Older racemes resist

FIG. 3.—A naturally infected palm, on which the young leaves have collapsed and fallen, leaving a crown of nearly horizontal leaves

decay, and nuts 3 to 4 inches in diameter usually remain on the tree until they mature, or until the supporting leaf falls, which frequently happens. The falling immature nuts, noted by Johnston as a characteristic symptom of the Cuban bud rot (*11*), has not been frequently observed in Porto Rico.

FIG. 4.—Advanced stage of bud rot, from natural infection. Most of the horizontal leaves have fallen

FIG. 5.—An infected bud, with the outer leaf bases removed, exposing the brown decayed penetration spot at the base of the leaf

FIG. 6.—The same bud as in Figure 5, with more leaf bases removed and portions cut away to show the infected interior tissues. Note the discoloration at the limits of the lower decayed area

FIG. 7.—Dead young leaves removed from the central column of an infected palm. The bases are completely disorganized into a soft, wet, malodorous mass of tissue

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Occasional cases of concentric rows or rings of spots on the leaf pinnae have been observed. Examination and isolated cultures usually revealed infection by *Pestalozzia palmarum* Cke. In a large majority of bud-rot cases spotting of the leaves did not occur. This fact would seem to be in keeping with the many cases of lateral penetration into the bud, whereas Reinking (*15*) found spotting of leaves and vertical penetration in the Philippines.

THE ORGANISM

The earliest attempts to isolate the causal organism of bud rot were made as follows: Half-inch cubes of tissue were removed from the margin of the rotted area of diseased buds. The cubes were surface-sterilized in a solution made by dissolving 1 gram of mercury

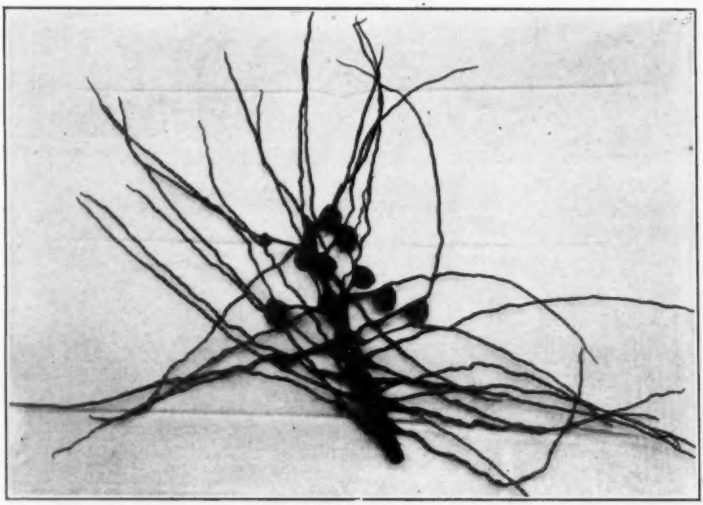

FIG. 8.—Blackened dead raceme and young nuts from a bud-rot-infected palm. The raceme was invaded and rotted at the base

bichloride in 500 c. c. of 50 per cent alcohol. After remaining five minutes in the disinfectant, the cubes were flamed and cut open with a scalpel. Bits of the interior tissue were then transferred to tubes of melted potato and beef agar, where they were crushed. The agar was then poured on plates. Abundant bacterial colonies appeared, nearly 90 per cent of which seemed to be identical. The results of inoculations with bacteria will be discussed later.

In February, 1923, during an examination of diseased buds from Tres Hermanos, a brown decayed spot, covered with white fluffy mycelium, was discovered on a leaf base. Cultures were obtained by transferring the mycelium directly to potato-dextrose agar, where typical Phytophthora colonies were obtained. Since that date dozens of diseased buds have been examined and in every case the same fungus has been isolated from the more fibrous portions of the bud. In very early cases of bud rot Phytophthora spores and mycelium were found between the folded pinnae of the dead young leaf.

The fungus grows well on potato-dextrose agar, the earliest growth being subsurface and flocculent in appearance, followed by a spreading growth on the surface, which is covered within a few days by a thick, white, cottony growth of aerial hyphae that disappears as the cultures become old. The surface of the agar is then covered by a light gray, thick, leathery, tough welt of mycelium. Conidia and chlamydospores are produced. No oospores have been found, although they have been searched for very frequently in the diseased buds and in cultures.

The aerial hyphae are colorless, branching, straight, smooth, and clear when young, and become densely granular with age, and nonseptate, except in occasional old hyphae. With age, large, slightly yellowish oil globules, equaling the hyphae in diameter, often appear in the filaments. They are spherical or oblong, sometimes filling the hyphae for a length several times exceeding their diameter. The aerial hyphae in mature cultures vary from 2.8 μ to 5.6 μ in diameter. Hyphae growing submerged in agar cultures are densely granular-crooked, gnarled, and swollen, especially at the branches. They are granular, usually contain numerous large and small oil globules, and vary from 3.2 μ to 12 μ in diameter. In tissues the mycelium is intercellular, entering the host cells by means of slender, unbranched haustoria.

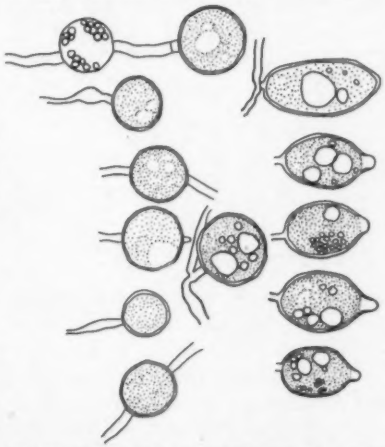

FIG. 9.—Conidia and chlamydospores of a small chlamydospored strain of *Phytophthora faberi* isolated from cases of coconut bud rot. × 300

Conidia are produced abundantly on sterile moistened corn meal, especially when there is not enough water added to permit of a luxuriant mycelial growth. They are oval to oblong, granular, often vacuolate, colorless or slightly yellow, and usually, but not always, prominently papillate with a colorless hyaline papilla (fig. 9). The spore wall is distinct and hyaline and may be continuous with the conidiophore, which is colorless, occasionally septate, 3 μ to 5 μ in diameter, and 50 to several hundred microns long. After a conidium is formed the conidiophore resumes growth in the same general direction by growing around the spore, or by pushing it aside. Another spore is formed and growth is again resumed. This may continue until one conidiophore has produced as many as 20 conidia. Germination is nearly always effected by the production of 1 to 10 or more germ tubes which emerge from any portion of the spore (fig. 10). Secondary conidia are occasionally formed by the growth of a conidiophore from the papilla, and usually are much smaller than is the primary spore which bears them. Germination by the formation of zoospores is rare. Eleven-day-old cultures of the fungus on corn meal, having abundant mature conodia, were placed in tap water, agitated frequently, and placed in strong light. One series of cultures was held at 27° C. During the first 4 hours the

suspension of spores was examined each half hour. Approximately 300 conidia were examined at each interval. The final examination was made after 22 hours. Not a single case of zoospore formation was observed, nor were there empty sporangia with the papilla broken off from which zoospores might have escaped. The second series was held at 20° C., as recommended by Gadd (*9*) for zoospore formation. In 1½ hours, one instance of zoospore formation was noted among 600 conidia examined. An empty sporangium was noted among 600 conidia which were examined after 2 hours. The last examination, made after 18 hours, showed no increase in the percentage of conidia becoming sporangia through the formation of zoospores. In both sets of cultures, germination of conidia and chlamydospores, as previously described, began in about 4 hours, and at the last examination a large percentage of them had produced germ tubes. Conidia from 8 to 10 day old cultures on corn meal vary in length from 26.3 μ to 88.5 μ, and in diameter from 17.8 μ to 41 μ. The average dimensions of 800 conidia are 52.67 μ by 30.95 μ.

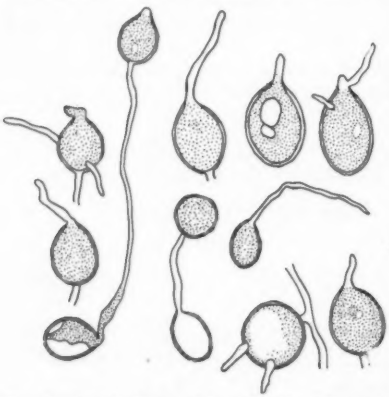

FIG. 10.—Germinating conidia and chlamydospores. Note the production of secondary forms following germination. Germ tubes are the usual means of germination. × 300

Chlamydospores are borne abundantly in potato-dextrose agar cultures that are grown in the light. They are spherical, lemon-yellow, densely granular, and often vacuolate, with a colorless hyaline wall (fig. 9). Those occurring in coconut tissues are somewhat irregular in shape, and dark in color, with a heavy yellow wall which is usually two to three times as thick as the wall of those grown in cultures. They are borne terminally or on short branches, which are, for the most part, perpendicular to the hyphae. Intercalary chlamydospores are also common. Germination occurs readily in the presence of moisture and 1 to 20 germ tubes may appear (fig. 10). No secondary chlamydospores have been noted arising from a germinating chlamydospore, although occasionally a chlamydospore is produced by a germinating conidium. No zoospore formation has been seen in chlamydospores. Those which were measured from 13-day-old potato-dextrose agar or corn-meal cultures were found to vary in diameter from 19.6 μ to 49.2 μ. Eight hundred chlamydospores average 34.8 μ in diameter.

Table I shows the frequency with which conidia and chlamydospores fall into length and diameter classes. The conidia are in two series, representing strains from two diseased buds. The chlamydospores also are in two series. Series I contains measurements of chlamydospores from 13-day-old potato-dextrose agar cultures; Series II, measurements from 13-day-old corn-meal cultures. The same strain was used in each series.

TABLE I.—*Dimensions of conidia and chlamydospores of Phytophthora faberi from rotted coconut buds*

Class (in microns)	Number of conidia in —				Number of chlamydospores in—	
	Series I according to—		Series II according to—		Series I according to—	Series II according to—
	Length	Diameter	Length	Diameter	Diameter	Diameter
15.5 to 17.49	0	0	0	0	0	0
17.5 to 19.49	0	0	0	2	0	0
19.5 to 21.49	0	0	0	2	0	5
21.5 to 23.49	0	8	0	3	9	4
23.5 to 25.49	0	10	0	2	18	2
25.5 to 27.49	0	34	2	18	30	10
27.5 to 29.49	1	93	1	56	56	15
29.5 to 31.49	1	115	2	94	54	15
31.5 to 33.49	1	104	2	138	81	44
33.5 to 35.49	4	27	2	56	42	55
35.5 to 37.49	2	7	1	18	41	53
37.5 to 39.49	10	1	1	11	29	83
39.5 to 41.49	12	1	4	0	17	49
41.5 to 43.49	41	0	9	0	13	49
43.5 to 45.49	37	0	18	0	6	8
45.5 to 47.49	43	0	22	0	2	8
47.5 to 49.49	35	0	44	0	2	0
49.5 to 51.49	40	0	36	0	0	0
51.5 to 53.49	60	0	63	0	0	0
53.5 to 55.49	31	0	32	0	0	0
55.5 to 57.49	25	0	29	0	0	0
57.5 to 59.49	16	0	30	0	0	0
59.5 to 61.49	11	0	20	0	0	0
61.5 to 63.49	9	0	16	0	0	0
63.5 to 65.49	7	0	15	0	0	0
65.5 to 67.49	2	0	10	0	0	0
67.5 to 69.49	2	0	13	0	0	0
69.5 to 71.49	7	0	5	0	0	0
71.5 to 73.49	2	0	7	0	0	0
73.5 to 75.49	0	0	4	0	0	0
75.5 to 77.49	0	0	3	0	0	0
77.5 to 79.49	0	0	6	0	0	0
79.5 to 81.49	1	0	0	0	0	0
81.5 to 83.49	0	0	0	0	0	0
83.5 to 85.49	0	0	1	0	0	0
85.5 to 87.49	0	0	0	0	0	0
87.5 to 89.49	0	0	2	0	0	0
89.5 to 91.49	0	0	0	0	0	0
Total	400	400	400	400	400	400
Average	50.4	30.23	54.94	31.68	32.96	36.71

From Table I it can be seen that the largest class of conidial lengths is 51.5 μ to 53.49 μ for both strains, and that the conidial diameters fall most frequently into the classes between 29.5 μ and 31.49 μ and between 31.5 μ and 33.49 μ. These measurements correspond closely with those obtained by Rosenbaum (*16*) and Reinking (*15*) for *Phytophthora faberi* Maublanc.

Considerable discrepancy may be noted between the diameters of chlamydospores which were grown on potato-dextrose agar and of those that were produced on corn meal, the most populous class advancing from 31.5 μ to 33.49 μ for the former to 37.5 μ to 39.49 μ for the latter medium, on which the measurements obtained are somewhat smaller than those recorded for *P. faberi* by both Rosenbaum (*16*) and Reinking (*15*). Delacroix and Maublanc (*6*) describe "oospores" (chlamydospores) 45 μ in diameter. The difference in chlamydospore measurements is believed to be insufficient to warrant the separation of the Porto Rico isolation as a new species.

Another criterion selected by Rosenbaum (*16*) for the separation of species is the ratio of the length to the diameter of the conidia. Table II records the frequencies with which the ratios fall into classes. The ratios recorded here were obtained from the conidial measurements of Table I.

TABLE II.—*Ratios of the length to the diameter of the conidia of Phytophthora faberi (Porto Rican isolation)*

Ratios (in microns)	Number of spores in—		Ratios (in microns)	Number of spores in—	
	Series I	Series II		Series I	Series II
1.05 to 1.14	1	0	2.25 to 2.34	4	9
1.15 to 1.24	3	3	2.35 to 2.44	3	5
1.25 to 1.34	5	5	2.45 to 2.54	2	3
1.35 to 1.44	36	22	2.55 to 2.64	0	5
1.45 to 1.54	92	71	2.65 to 2.74	0	2
1.55 to 1.64	87	77	2.75 to 2.84	0	0
1.65 to 1.74	65	58	2.85 to 2.94	0	0
1.75 to 1.84	45	52	2.95 to 3.04	1	0
1.85 to 1.94	31	38	3.05 to 3.14	0	0
1.95 to 2.04	8	19			
2.05 to 2.14	12	15	Total	400	400
2.15 to 2.24	5	16	Average	1.65	1.73

The ratios given in Table II show considerable similarity to those obtained by Reinking (*15*) in comparing *P. faberi* strains from coconut and cacao in the Philippines.

Evidence is accumulating to support the view that *P. faberi* Maublanc is composed of many morphological and physiological races which differ permanently. The strain under consideration in this paper is therefore designated a small-chlamydospored strain of *P. faberi* Maublanc. In another paper various strains of *P. faberi* attacking coconuts and cacao will be compared.

In ripe tomatoes, the fungus causes a soft watery rot in 48 hours by the dissolution of the lamellae. When the fruit is kept in a moist chamber it becomes covered with a profuse growth of snow-white mycelium bearing conidia and chlamydospores (fig. 11). Potatoes are rotted slowly when kept in a moist chamber following inoculation. No infection occurred in immature cacao pods which were wounded to a depth of 3 to 4 millimeters and inoculated. In another trial, the wounds were kept moistened constantly for 4 days but the fungus failed to demonstrate pathogenicity. A strain isolated from cacao in Trinidad and received from S. F. Ashby was used for a parallel series of inoculations. A brown rot spread rapidly from the wounds, and within 3 days the rotted areas averaged 5.5 cm. in diameter (fig. 12). These results are in accord with those obtained by Ashby (*2*) and with the fact that the Phytophthora disease of cacao has not been found in Porto Rico by Fawcett (*7*, *1913*), nor by the writer.

The fungus produced a profuse white cottony mass of mycelium on sterile moist cubes of healthy coconut bud tissue. Chlamydospores were abundant after 12 days, but conidia were rare; and there was no evidence of putrefaction, nor was there any of the odor which is characteristic of field cases of bud rot.

Green coconuts having a diameter of 3 to 7 inches were inoculated in the laboratory after the perianth scales were removed from one

side of the stem. The fungus was placed in the exposed depression, which was kept moistened daily with a few drops of sterile water. After 48 hours the tissue was visibly invaded and blackened, and after 6 days the entire stem end of the husk was blackened. The rotted areas on 10 inoculated nuts had an average diameter of 10.2 cm. (fig. 13). Control nuts having the scales removed and moisture added daily remained healthy. This type of infection was not observed in the field, but may occur on green husks left on the ground in the groves.

INOCULATIONS WITH PHYTOPHTHORA FABERI

Cultures for inoculation were prepared by growing the fungus on moist corn meal in liter flasks for 20 days. Sterile rain water was then added, and the flasks were shaken vigorously to dislodge the spores from the mycelium. The suspension was used after 24 hours, at which time the conidia and chlamy-dospores had germinated by germ-tube production. No zoospores were seen. This follows closely the procedure used by Reinking (*15*).

SERIES I.—Eight palms, 10 to 15 years old, were prepared for inoculation by removing from them the outer leaf bases and strainers

FIG. 11.—A ripe tomato and a potato wound inoculated with *Phytophthora faberi*, and held in a moist chamber for 72 hours. Conidia and chlamydospores are abundant in the profuse mycelial growth on the tomato

to expose the young, tender, white leaf base. A cup having a diameter of about 1 inch was made of grafting wax and fastened to the leaf base at the point of its attachment to the trunk (fig. 14). The leaf base formed one side of the cup. The water suspension of germinated spores and mycelium was poured into 6 cups, and sterile rain water into 2. These inoculations were made April 4, 1924, previous to the beginning of the rainy season. Forty-eight hours later the tissue in contact with the suspension was slightly brown. In 10 days a rotted area about 2 inches in diameter had developed on all inoculated palms. The checks showed no evidence of infection. At this time all the cups were removed.

The first symptom of disease appeared July 6, 1924, 81 days after inoculation, when the youngest leaf of one palm was observed to be brown. Six months after inoculation the four youngest leaves had turned brown and collapsed, and were hanging downward, a typical case of bud rot (fig. 15).

A second inoculated palm showed the characteristic symptoms 102 days after inoculation, and a third 187 days after inoculation. The other 3 palms remained healthy for six and one-half months, when their inoculated leaf bases were removed. In each case the fungus had failed to penetrate the underlying leaf base. Microscopic examination of the decayed areas revealed the presence of numerous chlamydospores. The check palms remained normal.

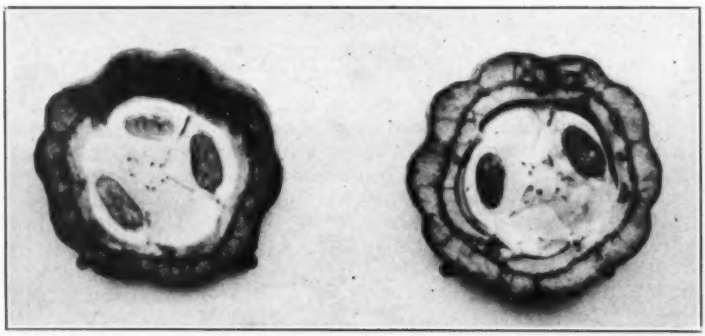

FIG. 12.—Left, section through cacao pod inoculated with a strain of *Phytophthora faberi* isolated from cacao in Trinidad. Right, section through cacao pod inoculated with a strain of *P. faberi* isolated from coconut bud rot in Porto Rico. Photographed 3 days after inoculation

SERIES II.—Eight palms, 7 to 15 years old, were prepared for inoculation as in Series I. Each was wounded to the heart of the bud, using for the purpose a $\frac{3}{16}$-inch auger. Grafting-wax cups were placed over the wounds. Inoculations were made April 4, 1924, by pouring a water suspension of the fungus into the cups on 6 palms as in Series I. Sterile rain water was again used for the control trees. After 10 days the cups were removed, the fungus having in-

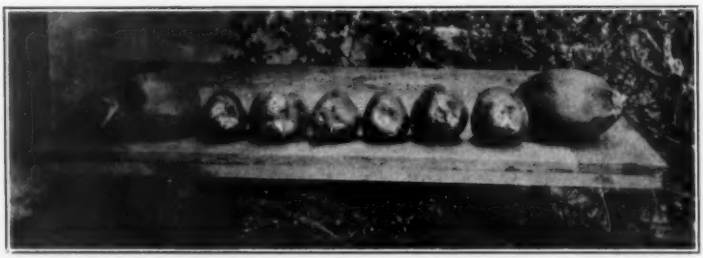

FIG. 13.—Green coconuts, 6 days after inoculation with *Phytophthora faberi*. The nut on the extreme left shows plainly the blackening of the husk due to invasion by the fungus

vaded the tissue about an inch around the wounds. In 24 days a brown liquid was exuding from each inoculated wound, emitting the odor characteristic of field cases of bud rot. When one of the palms was cut and the bud sectioned longitudinally, the growing point was found to be completely destroyed by a soft, watery, maladorous rot, indistinguishable in every particular from that occurring as a result of natural infection. The youngest leaf was dead, and mycelium

FIG. 14.—Young palm prepared for inoculation by removal of the outer leaves and the attachment of a cup made of grafting wax to receive the inoculating suspension

and spores of *Phytophthora faberi* were abundant between the folded pinnae. Symptoms which were typical of bud rot appeared in all 6 inoculated palms after periods of 24 to 80 days from inoculation.

The two check palms remained healthy for five months, when one exhibited symptoms of disease somewhat resembling those of bud

FIG. 15.—Bud rot produced by lateral inoculation of an unwounded palm with *Phytophthora faberi*. Photographed 6 months after inoculation

rot. A new leaf failed to emerge, and an older leaf gradually died back from the tip. One month later, no more leaves having died, the palm was cut and the bud dissected. A soft rot had destroyed the internal tissues, but had not invaded the young inclosing petioles and caused the death of those leaves, as is usual in typical bud-rot

cases. The offensive odor which is common in decayed buds was noted. No evidence of Phytophthora infection was found.

On October 8 a new shoot was observed pushing out laterally above the growing point on the other check palm. The leaves were dwarfed and distorted, but green and apparently healthy. The shoot emerged from the central column at an angle of about 45° (fig. 16). This

FIG. 16.—A deformed bud emerging laterally from the central column of a palm wounded but not inoculated. Note the crinkled pinnae and the dwarfing of the leaves. Photographed 173 days after wounding

abnormality is apparently identical with cases which were observed by Sharples and Lambourne (*17*).

SERIES III.—Three 10 to 15 year-old palms were prepared for inoculation as in Series I and II, except that fewer leaves were removed from them, and the inoculations were made on leaf bases in which the chlorophyll had developed, but which were still in an upright position. Inoculations were made on unwounded tissue as

in Series I and on the same date. In no case was there any evidence of penetration by the fungus, and the palms showed no symptoms of disease.

SERIES IV.—On June 30, 1924, during the rainy season, 8 palms, 10 to 20 years old, were inoculated by pouring the water suspension

FIG. 17.—Palm inoculated with *Phytophthora faberi* by pouring a water suspension of the fungus among the young, unfolding, unwounded leaves. Two older leaves were cut off to expose the central column to view. Photographed 121 days after inoculation

of the fungus among the young, unfolding leaves. The palms were not wounded in any way. Sixty-eight days later two palms had been killed, as was evidenced by the death of the emerging leaf (fig. 17). The other inoculated palms had rows of spots on the pinnae of emerging leaves. The spots were at first lemon-yellow in color, surrounded by a brown border, but as they increased in size the border became almost black and the interior bleached (fig. 18). The

infected tissue was thin, dry, and papery, and showed the presence of mycelium in welts. When the mycelium was transferred to media it gave cultures which were indistinguishable from the inoculating strain. Infection occurred and the spots were produced prior to the emergence of the leaves, resulting in rows of spots across the leaves, each pinna bearing a spot which was similar in size and shape to those of adjacent pinnae. After 108 days *Phytophthora faberi* was reisolated from two more palms showing typical symptoms of bud rot. The other four inoculated palms were continuing to produce spotted leaves.

Two of the positive cases were cut for examination, showing that infection of the growing point had occurred as a result of vertical

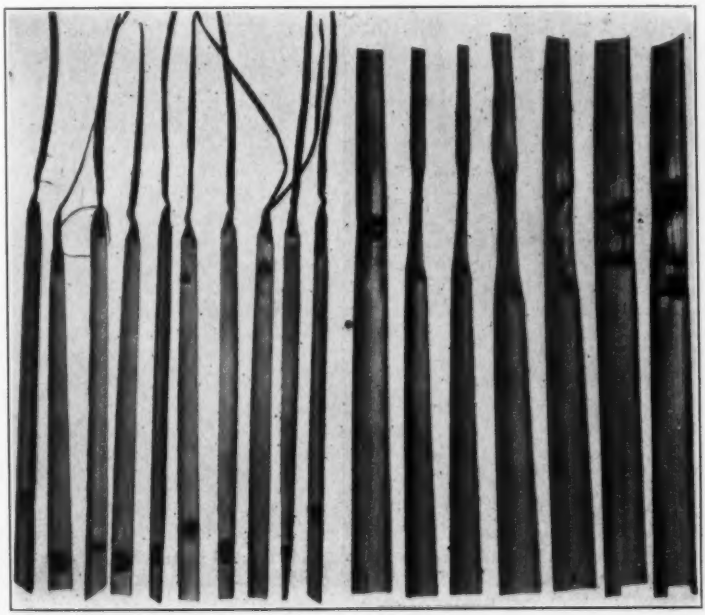

FIG. 18.—Spotted pinnae from an unwounded palm inoculated with *Phytophthora faberi* by pouring a water suspension among the young leaves

penetration. In one instance, a penetration spot was found on a leaf base, but the fungus had not penetrated the central "cabbage" through the intervening leaf bases.

Five months after inoculation the four remaining palms were producing healthy leaves and were thought to be free from infection, but on January 12, 1925, about six and one-half months after inoculation, the central leaf of one palm was found dead, and the bud decayed. Another palm was found to be infected on March 15, and still another on April 8, approximately nine months after inoculation. Dissection of the diseased buds showed lateral penetration of the fungus through the leaf bases. The original infection may have occurred on the leaf bases, since the fungus doubtless penetrates them slowly; or, the leaf-base infection may have been secondary, the infect-

ing material having been washed down from the primary infections on the leaf pinnae. *Phytophthora faberi* was reisolated from the infection spots in each palm.

ASSOCIATED BACTERIA

It has been recorded that the earliest attempts to isolate the causal organism of bud rot in Porto Rico gave nearly pure cultures of bacteria. The colonies were of two types, one which was round, white, shining, and convex; and the other, ameboid, thin, widely spreading, and somewhat opalescent. Pure cultures of each were replated, and each type produced the two types of colony, which were then regarded as variations within the strain. These colonies are very similar to those which are obtained from diseased coconut buds, and are described by Johnston (*11*). Insufficient studies were made on the organism to determine its exact identity. It is rod shaped, motile, and nongelatin liquefying. Some strains produce indol, others do not. Nitrates are reduced. Beef bouillon containing the organism is clouded rapidly with the formation of a slight pellicle and abundant flocculent sediment. Litmus milk reddens and coagulates. On potato plugs the growth is grayish, abundant, and shining; in coconut water the average gas production is 22 per cent, and the reaction of the medium is changed from +13.5 to +40, Fuller's scale; gas is produced in sucrose, dextrose, mannite, lactose, and glycerine; tender coconut tissue is decomposed and gives off a disagreeable odor which is identical with that found in coconut bud rot. No growth is produced in Cohn's solution. Uschinky's solution is moderately clouded, and a slight sediment is formed. In beef bouillon, made as recommended by Quirk and Fawcett (*13*), there is abundant growth in 48 hours within the reaction range of −20 to +30, Fuller's scale (P_H 5.2 to P_H 10.2), the first growth appearing at 5 hours in the +5 and +10 tubes (P_H 7.7 and P_H 7.2). All the above observations were made from cultures grown at room temperature (24° to 27° C).

INOCULATION EXPERIMENTS WITH BACTERIA

Series V.—On September 17, 1923, eight palms, each 7 to 10 years old, were inoculated by wounding them to the heart of the bud with a $\frac{3}{16}$-inch auger, and inserting in the wound about 8 c. c. of 48-hour bouillon culture of the organism described above. Sterile cotton was driven tightly into the wound to prevent the entry of insects.

Fifty days after inoculation a soft rot had caused the midrib of the emerging leaf of two palms to collapse (fig. 19.) The leaf was of a normal green color, both above and below the rotted point, which had the characteristic odor of bud rot. Within 82 days leaves showing evidence of wounding or of disease had pushed out from seven palms; in some cases a portion of the midrib had a black, crusty appearance; in others the leaf appeared to be deformed or dwarfed. In each case some of the pinnae exhibited a "cut" appearance, caused by decay prior to emergence. A palm with a collapsed midrib was cut for examination 81 days after inoculation. The leaf bases and strainers through which the auger had entered showed a rotted area about an inch in diameter around the wound. The collapsed midrib was rotted a few inches above and below the wound (fig. 20). A young leaf about 14 inches long was partly

decayed at the tip where wounded. The auger had entered slightly above the generative tissue, which showed no evidence of infection. Cultures that were obtained from the invaded leaves established the identity of the organism isolated with that used for inoculation.

The eighth palm ceased growth, no new leaves emerging. In seven months a young leaf died. No other leaves were affected. In 11 months, when the palm was felled, the old leaves were noted to be green and healthy. The bud was destroyed and had the characteristic odor of bud rot. The auger had entered the generative tissue.

Three check palms were wounded and injected with 8 c. c. of sterile bouillon. Two of them behaved exactly as did the inoculated palms, sending out deformed, dwarfed, or discolored leaves. One check produced a small leaf which soon died; the generative tissue

FIG. 19.—Palm wound inoculated with bacteria. The midrib of the young folded leaf has collapsed. This palm recovered, and showed no evidence of infection 10 months later. Photographed 50 days after inoculation

had been pierced and had decayed. Isolated bacteria appeared to be identical with those that were isolated from inoculated or naturally infected palms.

With the exception of the two that succumbed, each of the inoculated and check palms continued growth. As succeeding leaves appeared, the effects of the wounds were observed to be nearer the tips. In 11 months the new leaves were normal in size, vigor, and color.

SERIES VI.—The bacterium reisolated from an inoculated palm in Series I was used for the inoculation of twenty 15 to 20 year old palms on January 2, 1924. Fifteen palms were inoculated by pouring 50 c. c. of 48-hour bouillon culture into the heart of the plant between the emerging, unfolding leaves. Five palms were inoculated by cutting off the tip of an unopened flowering raceme sheath and pouring the culture on the young raceme. Ten months later no disease symptoms had appeared.

SERIES VII.—Ten palms of the same ages were inoculated as the 15 had been in Series VI, inoculation taking place June 6, 1924, during the rainy season. All inoculations have resulted negatively.

DISCUSSION OF THE INOCULATION EXPERIMENTS

All the described inoculations with *Phytophthora faberi* and the unidentified bacterium were made on the grounds of the agricultural experiment station, which is remote from any known case of coconut bud rot. Most of the inoculated palms were 10 to 20 years old; in some instances palms which were thought to be about 7 years old were used. All inoculations were made under field conditions, with no unnatural protection, and no moisture except the rains and the moisture used for the inoculations. The criticisms of Sharples and Lambourne (*17*) of bud-rot inoculation work in general do not apply to Series I, III, IV, and VII of the above-mentioned experiments. All records of inoculations as positive bud-rot cases are based upon

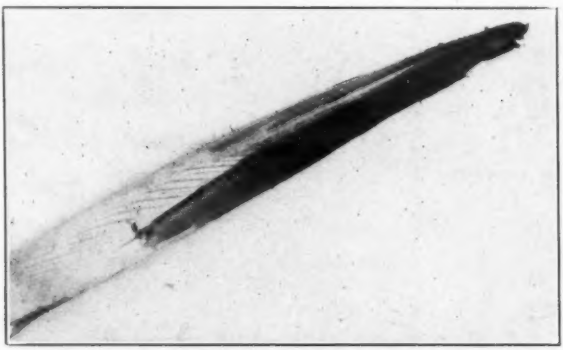

FIG. 20.—Wounded bud from a palm with a collapsed midrib very similar to Figure 17, showing the local decay caused by bacteria. In the interior of the bud a young leaf 14 inches long showed slight decay where wounded near the tip. A younger unwounded leaf was healthy. The rot showed no tendency to enter the growing point, and the palm probably would have recovered

the destruction of the growing point and not upon the wilting, browning, or collapsing of the central leaves only. During the period of observation not one case of disease was noted to occur among the numerous palms which were not included in the experiments.

The results recorded are believed sufficient to establish the infectious character of coconut bud rot as it occurs in Porto Rico, and to prove (1) that *Phytophthora faberi* Maublanc, or a subspecies, is the causal organism; (2) that the bacteria so abundant in rotted tissues are a secondary factor only, causing the decay of the heart tissues, and being incapable of invading unwounded buds; and (3) that wounding the generative tissue may cause the death of a palm by the introduction of omnipresent putrefactive bacteria.

The writer believes that this paper makes the first record of the production of the disease in the West Indies by the inoculation of unwounded palms with *Phytophthora faberi*. The appearance of typical bud-rot symptoms on seven of eight inoculated, unwounded palms within 282 days, the appearance of spotted leaves on the

remaining palm, and the reisolation of the organism from each inoculated palm, establishes the causal organism and its transmissibility.

The failure of the bacteria to attack unwounded trees under either dry or moist conditions, and their feeble pathogenicity to wounded leaf bases and midribs, eliminates the species as a causal factor. Their ability to break down coconut tissue with the evolution of the characteristic odor of bud rot indicates that they are an important factor in the destruction of the bud *after* the generative tissue has been injured or killed by another agent.

The death of two of five wounded uninoculated palms renders the positive results which were obtained by wounding somewhat uncertain, yet 100 per cent rapid, deaths following wound inoculations with *P. faberi;* while the constant presence of the fungus on the young dead leaves, compared with the slow dying observed in wounded uninoculated palms, certainly indicates pathogenicity.

The fatality of wounds seems to depend upon the carrying of bacteria into injured generative tissues. Since a wounding agent must penetrate several leaf bases, it is probable that each wound was infected. The writer has been reliably informed of a case in Florida where about 20 per cent of scrub palmettos (*Sabal megacarpa*) died when shot in the bud with a rifle.

Penetration of the bud by *Phytophthora faberi* may occur either laterally or vertically. In Series I, lateral penetration was produced under artificial conditions approaching as nearly as possible natural conditions. The broad leaf bases of the palm form a cup at the point of attachment to the trunk. Water may be found in the cup several days after heavy rains. The grafting-wax cups simulate this natural cup, and the course of infection following inoculations by this method is probably identical with the commonly found cases of penetration of laterally penetrated buds. Series IV resulted in vertical penetration of the bud, as is to be expected from pouring the inoculum between the youngest leaves. The preponderance of lateral penetrations in naturally infected cases may be due to the greater moisture retaining ability of the cupped leaf bases.

The appearance of concentric rows of Phytophthoralike spots on older leaves of palms bearing unmarked young leaves would seem to indicate that primary infections on the young leaves do not always result in infection of the growing point, as the rapid elongation of the emerging leaves tends to carry the infected tissues away from the generative tissue. However, the results of inoculations, obtained in Series IV when spotted leaves appeared soon after inoculation, followed by the emergence of healthy, unmarked leaves and finally by the death of the palm, indicate that infection probably occurred on the leaf bases, provided the spots really were due to Phytophthora infection, for the fungus could not be isolated from old leaf spots.

Lateral penetration is not accompanied by leaf-spotting. The experiments show that penetration of the leaf bases may cease before the bud is reached, the infected areas retaining the fungus in a dormant condition from which, no doubt, growth and penetration may be resumed under favorable conditions. Lateral penetration probably requires more time than does the vertical type, but is believed to be more dangerous under local conditions.

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MOISTURE AS A DISEASE FACTOR

The survey of the western coast was made at irregular intervals from November, 1923, to June, 1924. Palms showing the earliest symptoms of budrot were most numerous in November and December. The disease gradually decreased during the winter months until in April there were only a few palms showing the earliest symptoms. The rainy season extends from May to October, and the winter months are usually quite dry. The mean monthly precipitation, as recorded at Añasco and Mayaguez, is as follows:

Average monthly precipitation, in inches, as recorded at Añasco and Mayaguez

January	1. 57	May	9. 11	September	9. 64
February	2. 62	June	9. 23	October	10. 27
March	3. 46	July	10. 65	November	6. 35
April	5. 46	August	10. 57	December	2. 89

It is apparent that infection occurs mainly during the rainy season, since the symptoms appear near its end or early in the dry season. When the penetrating fungus has failed to enter the watery tissues of the heart or youngest leaves it probably remains dormant during the winter and becomes active again when moisture is abundant. The coolest and warmest months are February and September, with mean temperatures of 74.4° and 79.5° F., respectively. The fall in temperature during the winter is too slight to affect the progress of the disease.

New cases appearing during the dry season were usually those of palms which made rapid growth and were sheltered by houses or older palms. Such palms are easily penetrated laterally on account of their high moisture content.

The observations as to seasonal occurrence noted herein coincide with those of Ashby (*1*) and Butler (*4*) and others.

The western coast of Porto Rico, although short, has great variation in average annual precipitation, from aridity in the south to between 80 and 90 inches in the north-central region, and decreasing to 54 inches in the north. The survey shows that the limits of the bud-rot-infected area fall within the heavy rainfall area. High precipitation, at least during a few months of the year, seems to be necessary for the infection of the heart tissues of the palm.

Young palms in plantations of older palms have been found attacked in larger numbers than the older ones, due to the ease with which the fungus may be blown or washed down among their leaves, and to decreased evaporation caused by their sheltered location. Such palms retain free water following rains considerably longer than do the tall, exposed palms.

Plantations in low, poorly-drained situations are especially subject to attack. At first the possibility of physiological causes suggested itself; later, however, the appearance of the disease only within the last three to five years among such old plams in different poorly-drained localities indicated another cause. Examinations of early cases from these plantations revealed the constant presence of *Phytophthora faberi*.

DISSEMINATION

No direct experimental evidence has been obtained regarding the dissemination of bud rot. Observational evidence indicates that the wind is the principal agent. The dead young leaves are covered with mycelium and spores which, upon drying, are easily detached and carried away by the wind; falling in favorable locations on other palms they germinate when moistened, and infection may result. Butler (*5*) believes that less than 3 miles is the ordinary jump, and that 10 miles may be considered the maximum. His discussion relates to palmyra palms, but is believed to be equally applicable to the coconut. A dead young leaf which was brought into the laboratory had an abundant surface growth of hyphae and spores. After two weeks the spores had all fallen away, and fungi, principally *Pestalozzia palmarum* Cke., had overgrown the hygrophilous Phytophthora.

Insects may be an important factor in dissemination. Numerous dipterous insects are attracted to decaying buds and the rotted tissues literally swarm with larvae. Bees are usually found about healthy palms, but are repelled from diseased specimens by the odor of the rotted buds. The shothole borer almost immediately attacks diseased palms, but has not been observed in the soft tissues of the bud. Johnston (*11*) considered the turkey buzzard a possible disseminator. Rats infest coconut groves, but in Porto Rico the palms are usually banded with galvanized iron which protects them from rat invasion.

Nut pickers seem negligible agents, for they do not come in contact with diseased tissues when gathering the nuts.

Although no infected nuts have been found on infected palms, it is possible that the disease may be carried long distances and transmitted to the seedling from an infected husk.

CONTROL EXPERIMENTS

In India, Jamaica, and the Philippines, control measures have been directed mainly toward the destruction of diseased palms. The success of the method seems to have depended largely, according to Butler (*5*), upon the thoroughness with which diseased palms were identified and destroyed, and the care used in following up the initial removal of infected palms to prevent reinfection from palms in the incubation stage during the earlier campaign.

In November, 1923, an infected grove of 440 palms was selected for a control experiment. The grove was chosen (1) for its location, being fairly well isolated from other infected groves, and (2) for the diversity in age of the palms, which ranged in age from 4 or 5 to possibly 50 years. Sixty-two bud-rotted palms were felled, and the tops, after being removed about a foot below the leaves, were piled and burned. Kerosene was used to facilitate burning and to produce enough heat to penetrate the water-soaked buds.

Monthly inspections showed no new cases of bud rot until May, when six palms in the earliest stage of the disease were observed. Five of these were in a group of closely planted, sheltered palms about 6 years old, and each was within 30 feet of the area from which diseased palms had been removed in November. The sixth case was a tall palm which was growing about 50 feet from a previously

removed diseased one. It is probable that the fungus was present in these palms in November, remained dormant during the dry season, and resumed growth and penetration with the advent of rains in April. Fom May to November no new cases appeared.

It has been shown that the young dead leaves are covered with the fungus, and are an important source of inoculation. Control measures should, therefore, be timed to remove infected palms as soon as the disease is diagnosed by one experienced in searching for it. Monthly inspections should then be made. If this is impracticable, the inspections should be made and the diseased palms destroyed during November and December, and May and June.

It has been shown that the appearance of spots on the leaves may be an indication of infection. The palm should be destroyed whenever examination or cultures show that the spots on young leaves contain *P. faberi*. It is realized that a search for spotted emerging leaves would be entirely impracticable among tall palms, especially in regions where the palm is not of great value. Such a method of diagnosis would not be excessively expensive, however, especially in the case of palms in nurseries, in sections like Florida, for example, where the palm is of value either as a curiosity or as an ornamental, and in other places where laboratory facilities are available to permit its use. The removal of infected palms following the identification of the disease on leaf spots would protect against the most dangerous source of inoculum, which is the dead central leaf.

The presence of other susceptible hosts often plays an important part in devising control measures. The strain of *Phytophthora faberi* Maublanc, obtained from coconut bud rot in Porto Rico, has not been proved pathogenic to any other host under field conditions. Inoculations on cacao have resulted negatively. No inoculations have been attempted on other palms. Those palms which occur most frequently in or near coconut plantations are the Porto Rican royal (*Roystonea borinquensis*), and the corozo (*Acrocomia aculeata*). No cases of natural infection have been observed.

Further experiments will be necessary to determine the necessity of removing green husks from infected groves.

SUMMARY

Coconuts are an important secondary crop in Porto Rico.

Scattered cases of coconut bud rot appeared about 14 years ago in the region on the western coast between Mayaguez and Rincon. A survey in 1923–24 showed that there were more than 800 cases in that district.

The earliest symptom of bud rot is the wilting and death of the youngest leaf, followed by the falling of it and surrounding leaves, leaving the palm without a central column of green leaves. The young tissues and the growing point are attacked and reduced to a watery, malodorous mass of rotted material. The old leaves fall away slowly until the trunk is completely defoliated.

Infection occurs by the penetration of the disease into the growing point, laterally through the leaf bases, or vertically in the central column of leaves.

The causal organism is a small chlamydospored strain of *Phytophthora faberi* Maublanc. The conidia have an average length and

diameter of 52.67 μ by 30.95 μ. The average ratio of length to diameter is 1.69. The chlamydospores have an average diameter of 34.83 μ. The fungus does not infect cacao pods.

Six unwounded palms were inoculated laterally on tender leaf bases. Three succumbed to typical bud rot.

Six wounded palms were inoculated laterally. All exhibited symptoms of bud rot and died.

Three unwounded palms were inoculated laterally on tough leaf bases. No infection resulted.

Eight unwounded palms were inoculated by pouring a water suspension of the fungus among the young leaves. Seven succumbed to bud rot.

The fungus was reisolated from each positive inoculation.

The incubation period of the disease varies from three to nine months or longer.

Two of the five wounded, uninoculated palms died from a disease resembling bud rot. Another produced a distorted lateral bud.

Rotted tissues contain enormous numbers of bacteria, of which pure cultures were obtained. No identification was attempted.

Eight wounded palms were inoculated laterally with pure cultures of the bacterium. Local decay resulted from injuries to and infection of young leaves. One palm, whose growing point was wounded, died. All others recovered. The organism was recovered from a local decayed area and used to inoculate 30 unwounded palms during the dry and wet seasons. All results were negative.

Palms attacked by *Phytophthora faberi* Maublanc exhibit the earliest symptoms of bud rot most frequently in November and December, at the end of the rainy season. The fungus may remain dormant in the leaf bases during the dry season.

The infected area lies in a region the annual rainfall of which is 80 to 90 inches.

Young, protected palms and those in low moist situations are especially susceptible.

The wind and perhaps insects are the principal disseminating agents.

In a grove of 440 palms 62 bud-rotted palms were destroyed to determine the effectiveness of eradication as a control measure in Porto Rico. In six months six new cases appeared, and these trees were destroyed. Six months later no new cases had developed.

The disease had not been observed on any other palms.

LITERATURE CITED

(1) Ashby, S. F.
1920. Notes on two diseases of the coco-nut palm in Jamaica caused by fungi of the genus Phytophthora. West Indian Bul. 18: 61–73.

(2) ———
1921. Relation between cacao pod rot and coco-nut bud rot. Agr. News [Barbados] 20: 318.

(3) Burger, O. F.
[1923]. Report of plant pathologist. Fla. Agr. Exp. Sta. Ann. Rpt. 1923: 52R–102R.

(4) Butler, E. J.
1910. The bud-rot of palms in India. Mem. Dept. Agr. India Bot. Ser. 3: 221–280, illus.

(5) Butler, E. J.
1918. fungi and disease in plants. an introduction to the diseases of field and plantation crops, especially those of india and the east. 547 p., illus. Calcutta and Simla.
(6) Delacroix, G., and Maublanc, C.
1909. les maladies des plantes cultivées dans les pays chauds. maladies du cacaoyer. i. la pourriture brune des cabosses. Agr. Prat. Pays Chauds 9 (2): 314–318, illus.
(7) Fawcett, G. L.
1911–14. report of the plant pathologist. Porto Rico Agr. Exp. Sta. Ann. Rpt. 1910: 35–36, 1911; 1911: 37–39, 1912; 1912: 31–33, illus., 1913; 1913: 26–29, 1914.
(8) Fawcett, W.
1889. report on the cayman islands. Bul. Bot. Dept. Jamaica 11, 8 p.
(9) Gadd, C. H.
1924. the swarming of zoospores of phytophthora faberi. Ann. Bot. [London] 38: 394–397.
(10) Hartley, C., and Rands, R. D.
1924. plant pathology in the dutch east indies. Phytopathology 14: 8–23, illus.
(11) Johnston, J. R.
1912. the history and cause of the coconut bud-rot. U. S. Dept. Agr., Bur. Plant Indus. Bul. 228, 175 p., illus.
(12) McRae, W.
1923. i. history of the operations against bud-rot of palms in south india. ii. inoculation experiments with phytophthora palmivora butl. on borassus flabellifer linn. and cocos nucifera linn. Mem. Dept. Agr. India Bot. Ser. 12: 21–70, illus.
(13) Quirk, A. J., and Fawcett, E. H.
1923. hydrogen-ion concentration vs. titratable acidity in culture mediums. Jour. Infect. Diseases 33: 1–59, illus.
(14) Reinking, O. A.
1919. phytophthora faberi maubl.: the cause of coconut bud rot in the philippines. Philippine Jour. Sci. 14: 131–151, illus.
(15) ———
1923. comparative study of phytophthora faberi on coconut and cacao in the philippine islands. Jour. Agr. Research 25: 267–284, illus.
(16) Rosenbaum, J.
1917. studies of the genus phytophthora. Jour. Agr. Research 8: 233–276, illus.
(17) Sharples, A., and Lambourne, J.
1922. observations in malaya on bud-rot of coco-nuts. Ann. Bot. [London] 36: 55–70, illus.
(18) Shaw, F. J. F., and Sundararaman, S.
1914. the bud rot of coconut palms in malabar. Agr. Jour. India 9: 111–117, illus.
(19) Tucker, C. M.
1924. la pudrición del cogollo del cocotero en puerto rico. Rev. Agr. Puerto Rico 12: 385–390, illus.
(20) ———
1924. controlling coconut bud rot. Porto Rico Agr. Exp. Sta. Agr. Notes 8, 1 p.

AN ALTERNATE EXTRACTION AND EXPOSURE METHOD USED IN CONNECTION WITH STUDY OF ARSENICALS[1]

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In a study of certain phases of the action of arsenicals it appeared that in the method here outlined, which may be designated the "alternate wetting and drying" or "extraction and exposure" method, there are possibilities both for a fundamental study of various factors involved in the action of insecticides and for guidance in the practice of preparing arsenical mixtures.

Briefly, the method is this. The determination of water soluble arsenic is made according to the usual procedure, or in the proportions used in preparing the tank mixtures for field work. The material is filtered for the purpose of determining the quantity of arsenic present, and the total residue is transferred to the filter paper and washed twice. When sufficiently dry for convenient handling, the residue is placed in a Petri dish, where it is spread out uniformly in a thin layer and exposed to the atmosphere. After a suitable period of exposure, which may range from a few hours to several days, the material is again extracted and the determination of water soluble arsenic made as before. This process of extraction followed by exposure, is repeated several times, the number depending on the particular phase to be studied and the data obtained from successive determinations.

Although this method can not be said to be a reproduction of conditions operative in field practice, it is certainly a closer approximation to such conditions than the single extraction and determination method generally employed for testing arsenical mixtures. It is quite reasonable to assume that with this method the general direction of the mode of action of the mixture in the field will not diverge significantly from that observed in the laboratory.

The proportions of water to residue used in the extractions following the first one should also possibly be related to some field conditions, such as the average ratios of dew and arsenical noted on leaves that have been dusted or sprayed. A laboratory imitation of this condition may, at least provisionally, be accepted as a basis.

The results of determinations made by several methods are given in Table I.

In Table I the first determination represents the usual single extraction method. The percentages found in the subsequent determinations with the alternate extraction and exposure method are remarkably high. It must also be borne in mind that in the latter case they are additive, the water soluble arsenic from the preceding extraction having been removed before the following extraction was made.

That it is this succession of wetting and drying or extraction and exposure, and not simply a prolonged contact of the mixture with water, that is instrumental in bringing about the high percentages of water soluble arsenic, is shown from the figures for sample 2 in Table

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TABLE I.—*Water soluble arsenic oxide (As_2O_5) in "dry mix," [a] by various methods (expressed in percentage of lead arsenate present in the mixture)*

Determination	Number of days elapsed since preceding determination	Sample 1—Alternate extraction and exposure method	Sample 2	
			Continuous extraction method [b]	Alternate extraction and exposure method
First		0.03	0.07	0.07
Second	7	1.34		
Do	4		.29	1.94
Third	9	1.54		
Do	6		.25	1.66
Fourth	7	1.83		
Do	5		.20	1.71
Fifth	7	1.66		
Do	6		.29	2.26

[a] In the "dry mix," the lime and sulphur were mixed dry and then added to the water.

[b] In this test the sample was kept in contact with the liquid during the whole time in which the alternate extraction and exposure test was carried on; for analysis, the mixture was thoroughly shaken and a portion immediately thrown on the filter, so the proportions of the ingredients remaining in the flask would not be much altered.

I. With this sample a continuous extraction was made on a different portion of the mixture concurrently with the alternate extraction and exposure method; water soluble arsenic determinations were made at various intervals and at the same time from each of the two portions. It will be noted that beginning with the second determination the portion submitted to continuous extraction showed hardly any variation, the percentages being very low throughout the period, whereas with the alternate extraction and exposure method, all determinations after the first show very high percentages of water soluble arsenic.

Mixtures which on the basis of the single extraction method would be pronounced safe, have been found by the method here proposed to behave at a subsequent stage in such a way as to make the danger of foliage injury almost a certainty. That similar observations have been made in actual field practice is well known, and may serve as a further illustration of how properly conducted laboratory tests may be helpful in the choice of mixtures intended for field use.

It is apparent that, as suggested by a number of workers, an atmospheric agent, probably carbon dioxide, acting upon the lime in the mixture, increases the water soluble arsenic. A mixture of sulphur and lead arsenate, in the usual proportions, to which was added calcium carbonate instead of hydrated lime, showed about 7 per cent of water soluble arsenic from the beginning.

The change of properties in arsenical mixtures with wetting and drying may bear some relation to such qualities as adhesion and spreading which should be studied independently or in connection with solubility. By devoting particular attention to a study of the mixture at that stage of the alternate extraction and exposure procedure at which a change, favorable or otherwise, takes place, a clue may be found which will lead to desirable adjustment of the mixture.

The method can, of course, be used also in combination with the study of such factors as varying degrees of atmospheric moisture, light and shade, and temperature.